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Imamura et al.

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(54) **HEPARIN-BINDING PROTEINS MODIFIED
 WITH SUGAR CHAINS, METHOD OF
 PRODUCING THE SAME AND
 PHARMACEUTICAL COMPOSITIONS
 CONTAINING THE SAME**

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C07K 14/475 (2006.01)

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(58) **Field of Classification Search** **514/8; 530/395, 396, 399, 402, 404, 411, 408**
 See application file for complete search history.

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(57) **ABSTRACT**

A heparin-binding protein functionalized by covalently bonding thereto a sugar chain, a method for producing the protein and a pharmaceutical composition containing the protein as an active ingredient, as well as a method for functionalizing a natural protein having no sugar chain by covalently bonding thereto a sugar chain.

5 Claims, 9 Drawing Sheets

FIG. 1

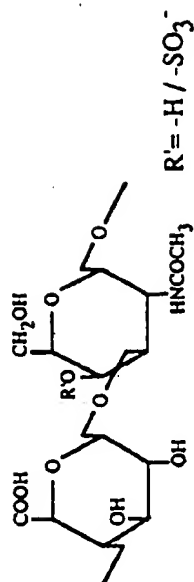
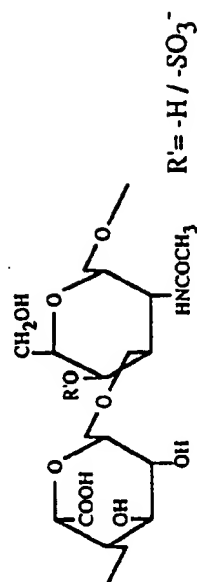
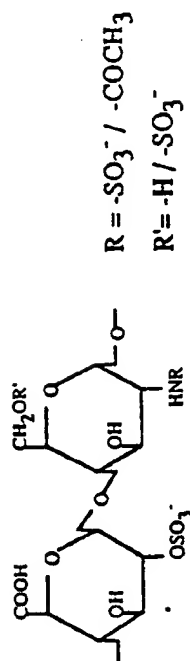
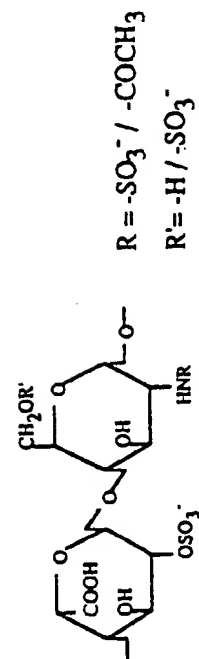
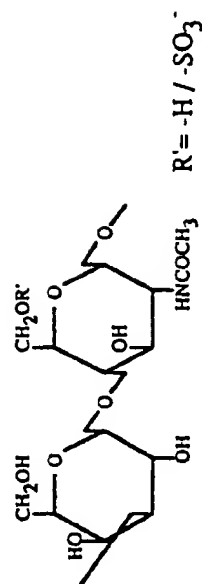
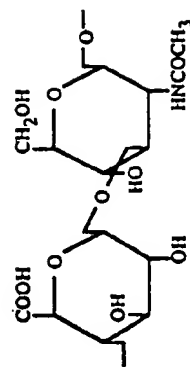
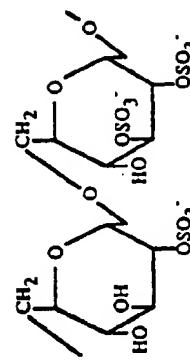
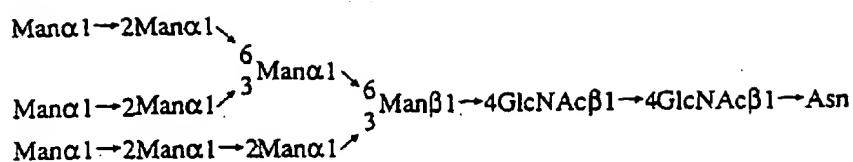
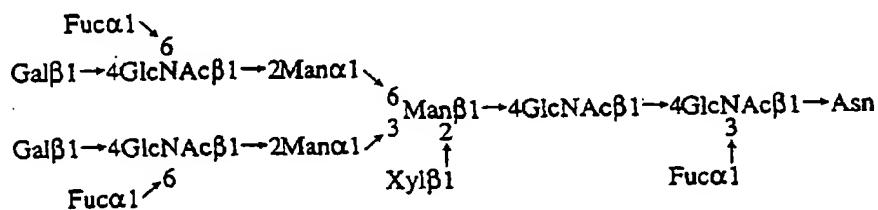
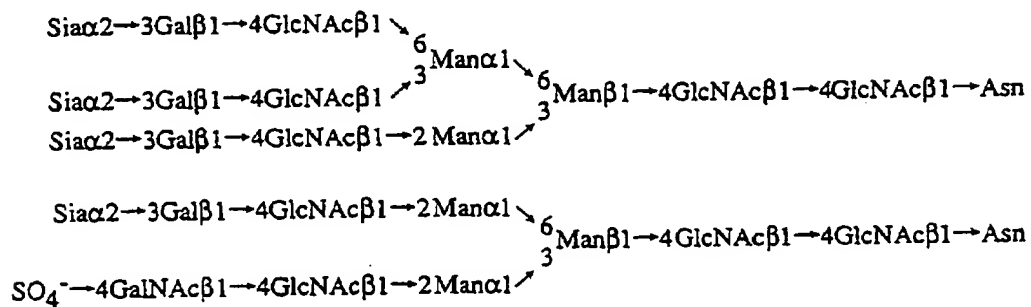
1) Chondroitin sulfate/(GlcA-GalN)_n2) Dermatan sulfate/(IdoA/GlcA-GalN)_n3) Heparan sulfate/(GlcA/IdoA-GlcN)_n4) Heparin/(IdoA/GlcA-GlcN)_n5) Keratan sulfate/(Gal-GlcN)_n6) Hyaluronic acid/(GlcA-GlcN)_n7) Dextran sulfate/(Glc-Glc)_n

FIG. 2

1) High Mannose Type



2) Complex Type



3) Hybrid Type

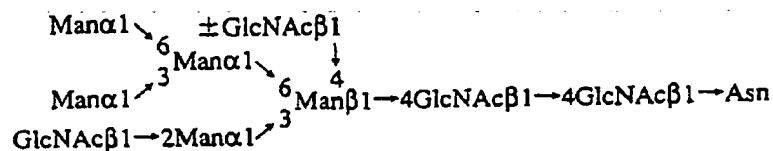


FIG. 3

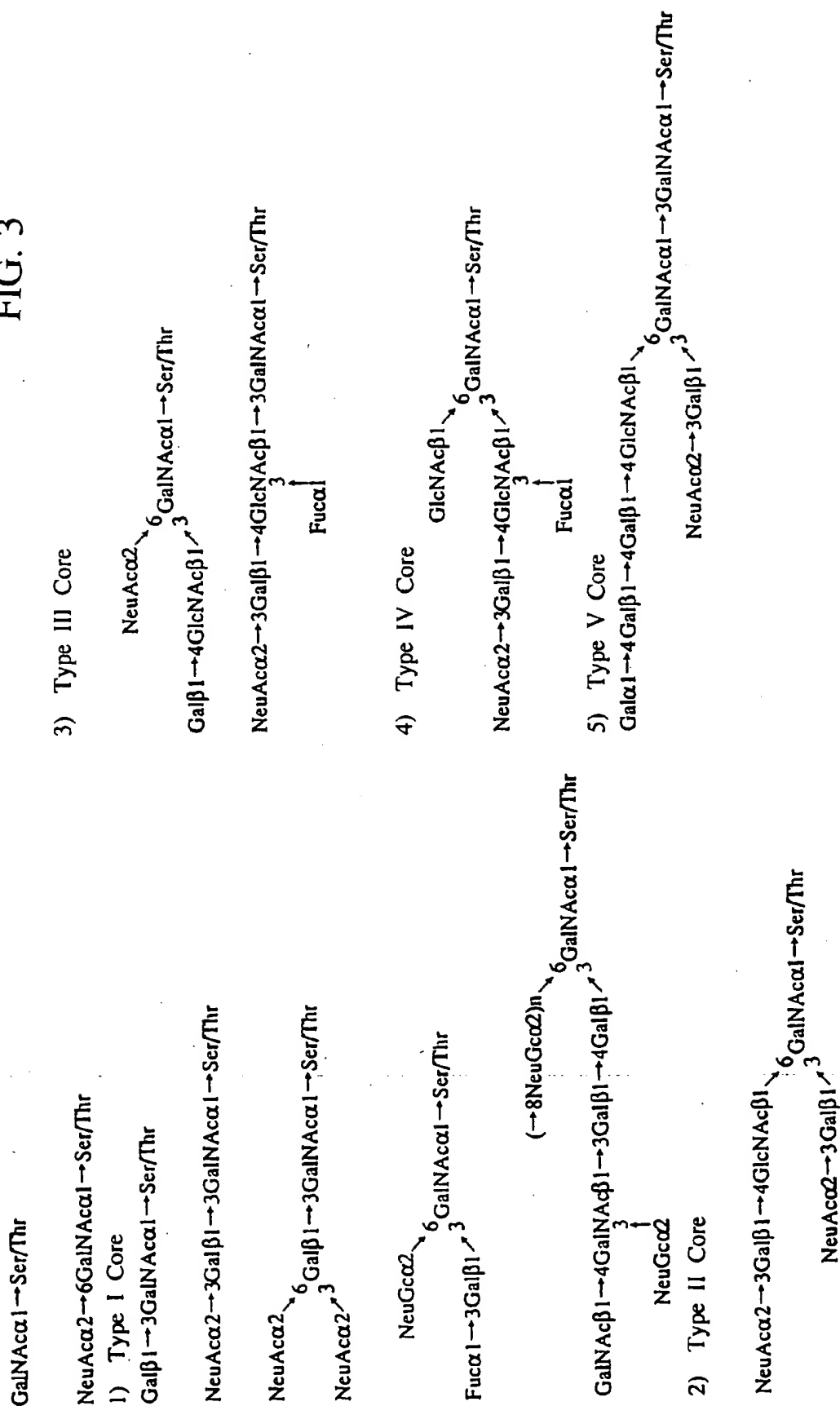


FIG. 4

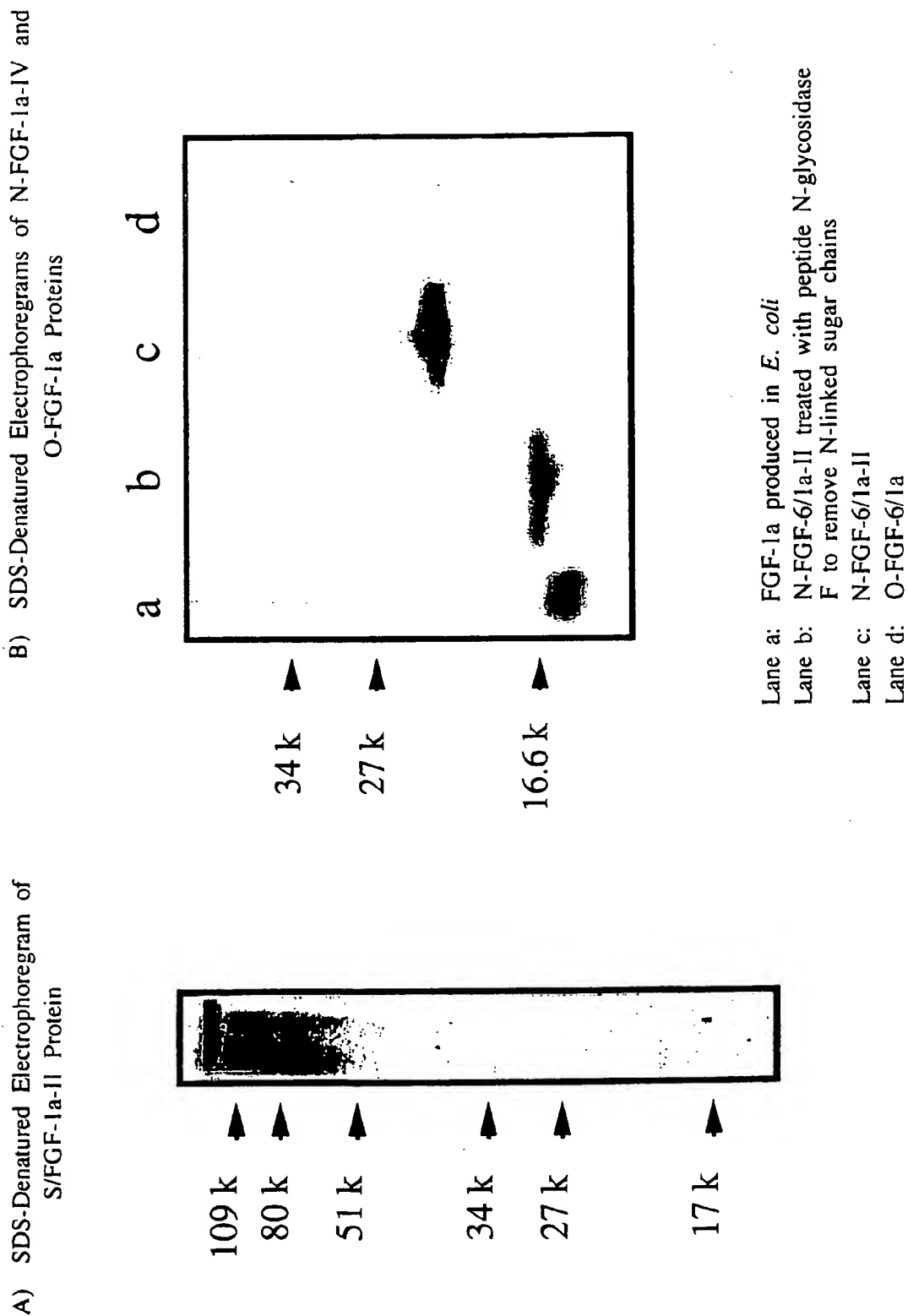
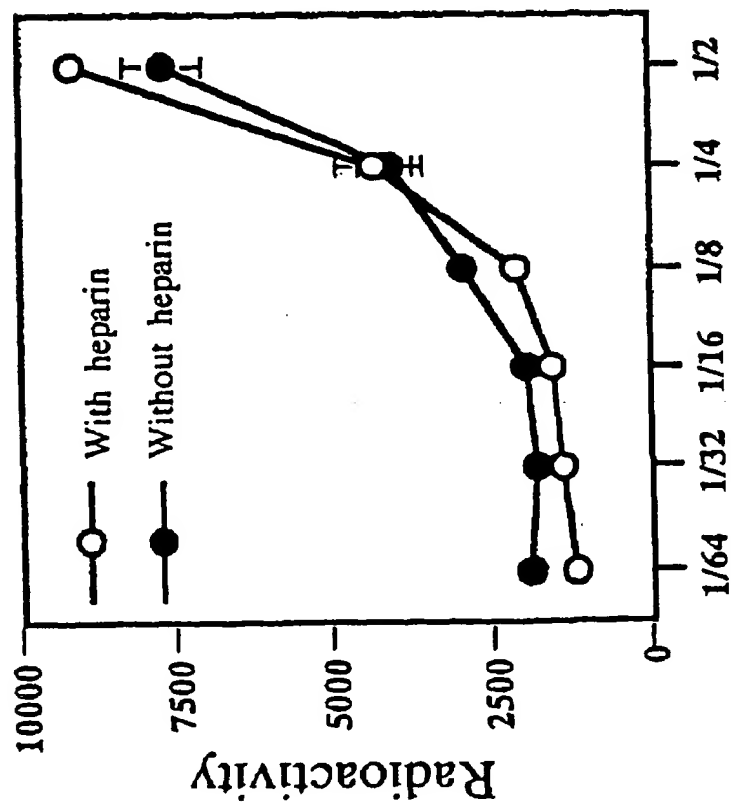


FIG. 5A

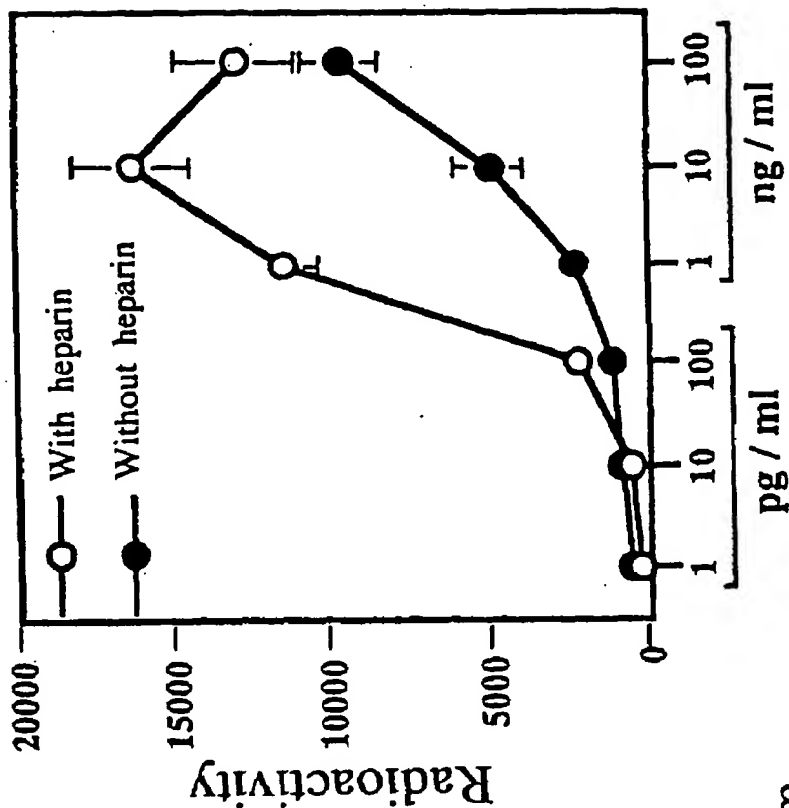
S/FGF-1a-II



Dilution of Conditioned Medium

FIG. 5B

E. coli-Derived FGF-1a



Concentration of
E. coli-Derived FGF-1a

FIG. 6A

S/FGF-1a-II

Without heparin
With heparin

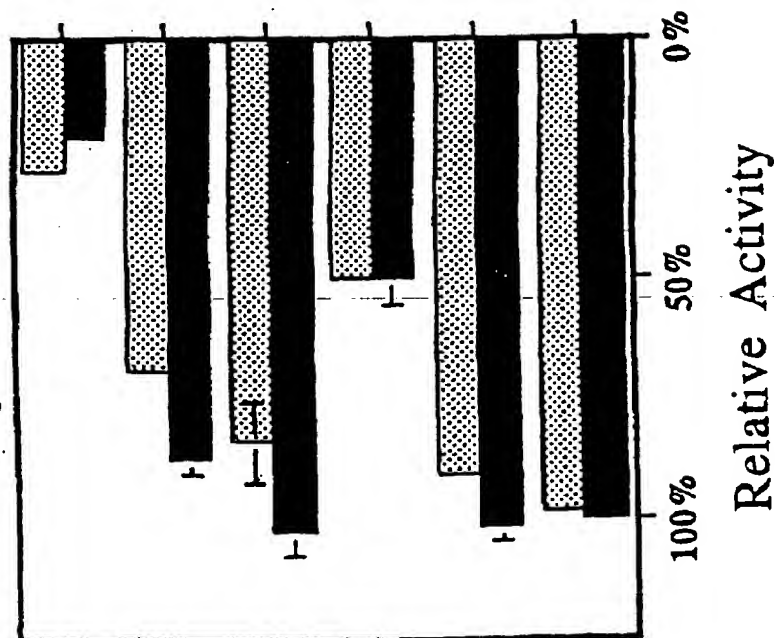


FIG. 6B

E. coli -Derived FGF-1a

Without heparin
With heparin

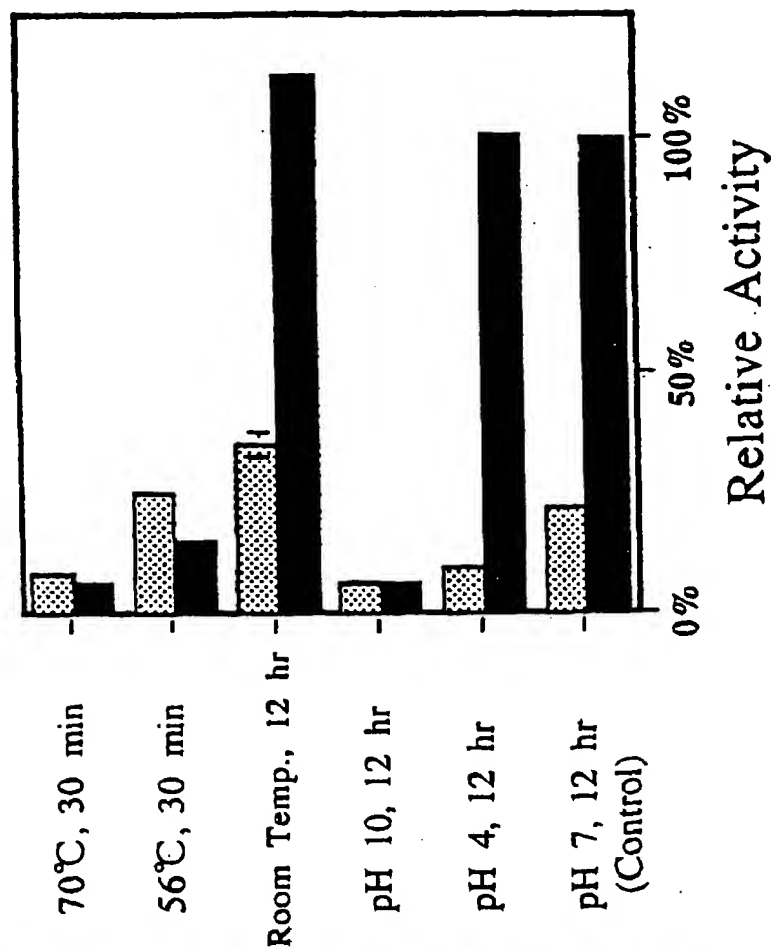


FIG. 7

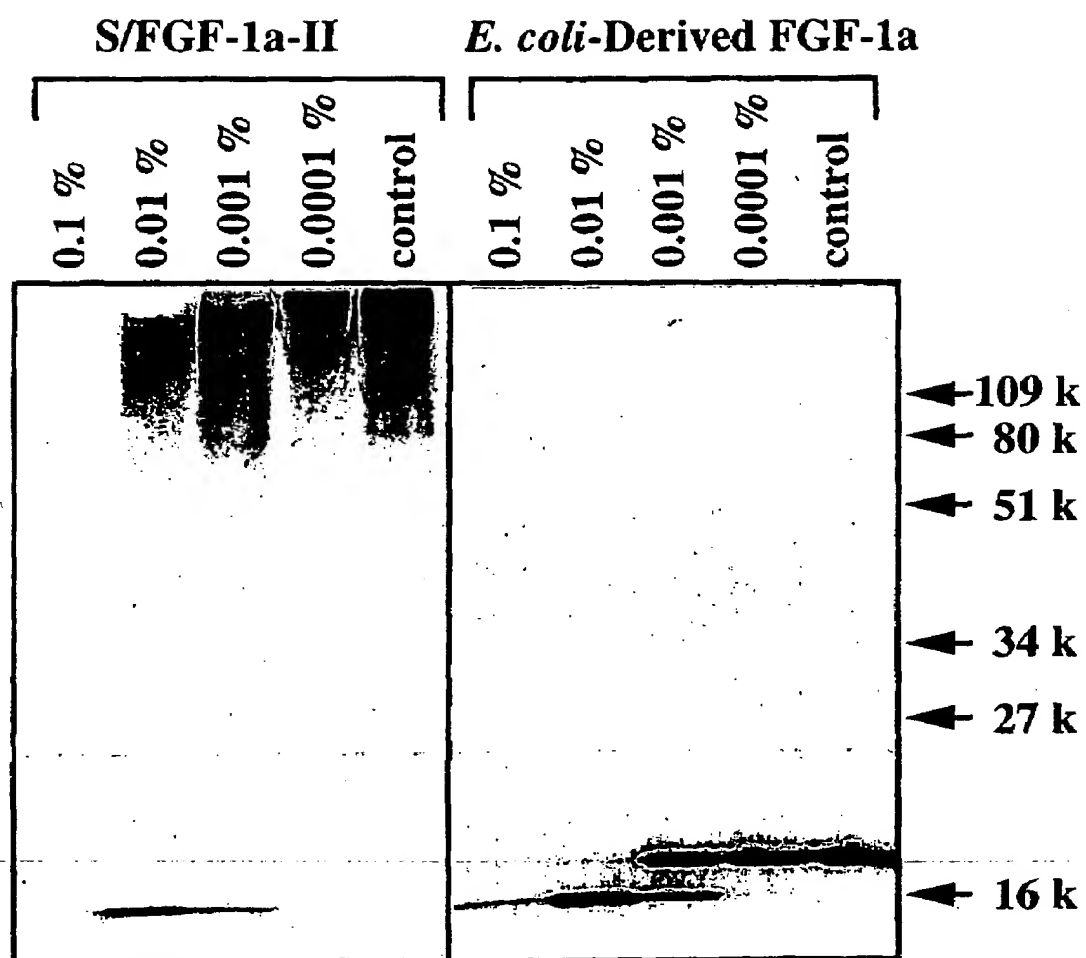
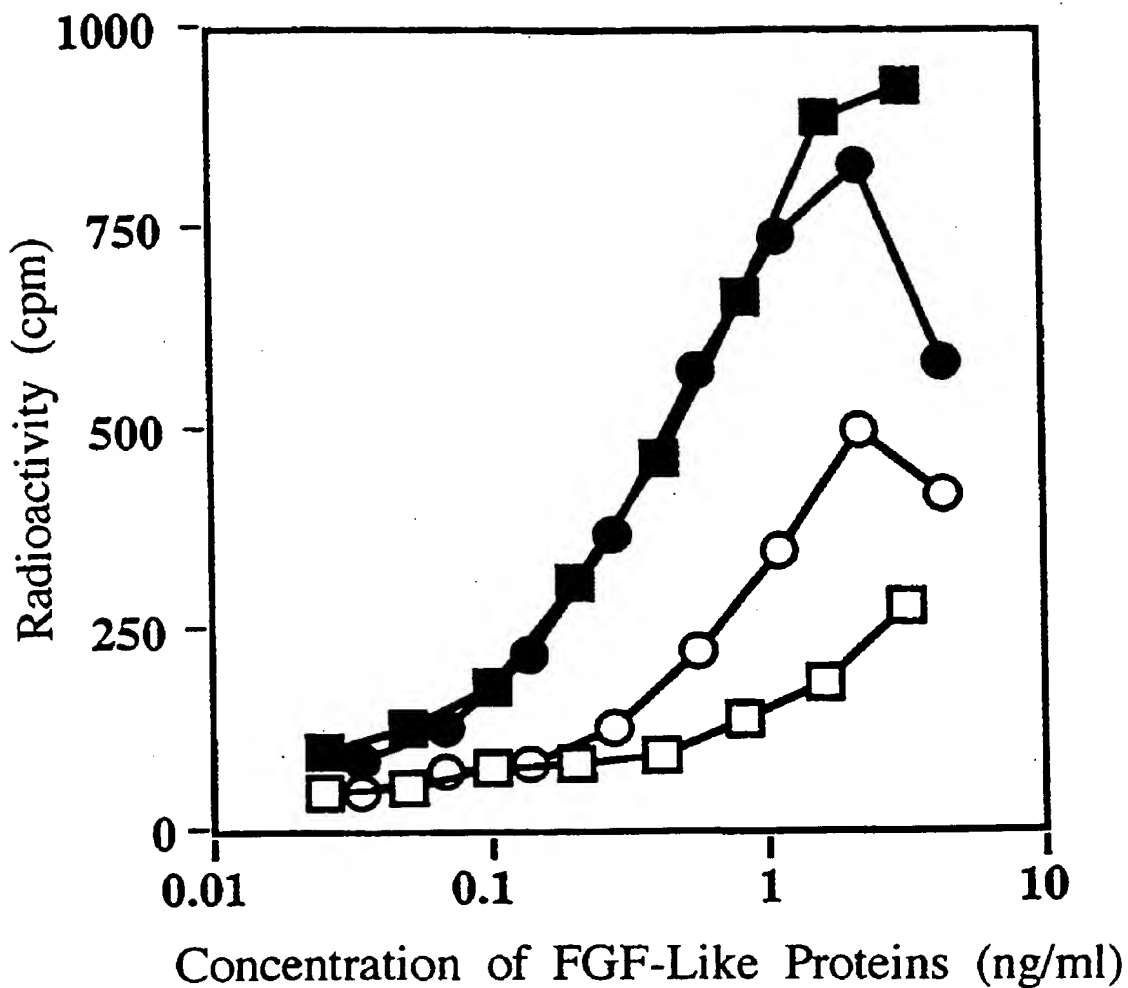
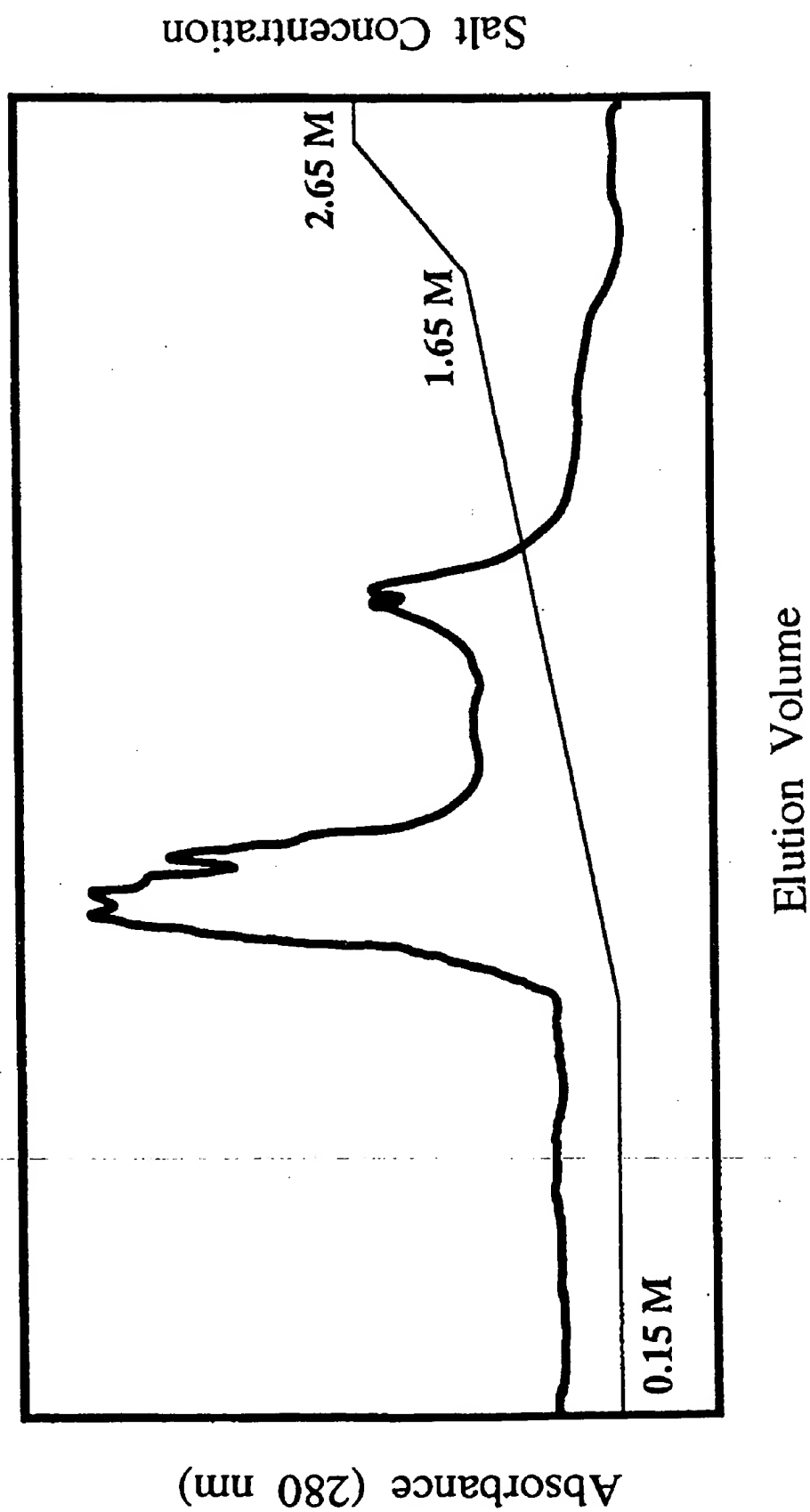


FIG. 8



- *E. coli*-Derived FGF-1a/with heparin
- *E. coli*-Derived FGF-1a/without heparin
- N-FGF-6/1a-IV/with heparin
- N-FGF-6/1a-IV/without heparin

FIG. 9



HEPARIN-BINDING PROTEINS MODIFIED WITH SUGAR CHAINS, METHOD OF PRODUCING THE SAME AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

BACKGROUND OF THE INVENTION

The present invention relates to a heparin-binding protein functionalized by covalently bonding thereto sugar chain(s), a method for producing the protein and a pharmaceutical composition containing the protein.

It has been known that heparin-binding proteins, among all, those proteins classified into the fibroblast growth factor (hereinafter, referred to as "FGF") family and fibroblast growth factor homologous factors strongly bind to heparin and heparan sulfate (sulfated polysaccharides) by a non-covalent bond. It has been also known that when a heparin-binding protein such as fibroblast growth factor is mixed with a sulfated polysaccharide such as heparin, the biological activity and physical properties of the heparin-binding protein are altered to change its function; sometimes, such a heparin-binding protein may acquire higher function. However, even if a sulfated polysaccharide was mixed with, the expected functionalization of the protein has been limited. Besides, when such a mixture is used as a pharmaceutical composition, unfavorable physiological activity attributable to a free sulfated polysaccharide has caused a problem. To date, there has been reported no protein in which a heparin-binding protein is joined with sulfated polysaccharide(s) by a covalent bond for the purpose of functionalization of the heparin-binding protein.

In addition, it has never been known to date that artificial addition of asparagine-linked sugar chain(s) (hereinafter, referred to as "N-linked sugar chain(s)") or serine/threonine-linked sugar chain(s) (hereinafter, referred to as an "O-linked sugar chain(s)") to a heparin-binding protein, particularly a protein of the FGF family or a fibroblast growth factor homologous factor, by covalent bond(s) can functionalize the protein. Furthermore, the general effect which N-linked sugar chain(s) or O-linked sugar chain(s) could give has not been known. Exceptionally, with respect to FGF-6, the role of the N-linked sugar chain(s) it naturally has was suggested in an in vitro translation system, but has not been proved directly. To date, there has been reported no example of joining a heparin-binding protein with N-linked or O-linked sugar chain(s) by covalent bond(s) for the purpose of functionalizing the heparin-binding protein.

It is an object of the present invention to improve the function of heparin-binding proteins. It is another object of the invention to establish a heparin-binding protein to which sugar chain(s) are covalently bonded and a method for producing the protein. It is still another object of the invention to provide a pharmaceutical composition containing the above protein.

SUMMARY OF THE INVENTION

The present inventors have made intensive and extensive researches toward the solution of the above problems. As a result, the inventors have noted the fact that sulfated polysaccharide(s), glycosaminoglycan(s), N-linked sugar chain(s) and O-linked sugar chain(s) are individually synthesized in living animal bodies as sugar chain(s) of a glycoprotein. Then, the inventors have found that it is possible to produce a heparin-binding protein having in its molecule sulfated polysaccharide(s), glycosaminoglycan(s), N-linked sugar

chain(s) or O-linked sugar chain(s) covalently bonded thereto by ensuring that a cDNA coding for a peptide to which any of the above sugar chains can be added is ligated to a cDNA coding for the heparin-binding protein, and by then allowing an animal cell to produce the gene product of the ligated cDNA. Furthermore, the inventors have confirmed that the function of the resultant sugar chain(s)-added heparin-binding protein is improved. Thus, the present invention has been achieved based on these findings.

The present invention provides a heparin-binding protein functionalized by covalently bonding thereto sugar chain(s). The sugar chain(s) may be selected from the group consisting of sulfated polysaccharide(s), glycosaminoglycan(s), N-linked sugar chain(s), O-linked sugar chain(s) and a combination thereof. The heparin-binding protein may be a factor belonging to the FGF family or its allied factor. The heparin-binding protein may be covalently bonded to the sugar chain(s) through a peptide to which the sugar chain(s) can be added. For example, the heparin-binding protein to which the sugar chain(s) are to be covalently bonded may be the following (a) or (b):

(a) a protein consisting of the amino acid sequence of SEQ ID NO: 1, 3, 5, 17, 19, 21, 23, 25, 27 or 29;

(b) a protein which consists of the amino acid sequence of SEQ ID NO: 1, 3, 5, 17, 19, 21, 23, 25, 27 or 29 having deletion, substitution, addition or modification of one or several amino acids, which has FGF activity and to which the sugar chain can be added.

In the heparin-binding protein of the invention, the sugar chain(s) may be bonded to the heparin-binding protein at a site forming a turn in the secondary structure or a site near one of the ends, or a site which would not change the tertiary structure of the protein greatly by addition of the sugar chain(s).

The present invention also provides a method for producing a heparin-binding protein functionalized by covalently bonding thereto sugar chain(s), comprising the following steps:

(a) a step in which a cDNA coding for a peptide to which sugar chain(s) can be added is ligated to a cDNA coding for a heparin-binding protein;

(b) a step of incorporating the resultant ligated cDNA into an expression vector;

(c) a step of introducing the expression vector into a host cell having sugar chain(s) addition pathway; and

(d) a step of expressing in the host cell a heparin-binding protein to which sugar chain(s) are covalently bonded through the peptide to which the sugar chain(s) can be added.

When the sugar chain(s) are sulfated polysaccharide(s) or glycosaminoglycan(s), the peptide to which the sugar chain(s) can be added may be a proteoglycan core protein or a part thereof. When the sugar chain(s) are N-linked sugar chain(s), the peptide to which the sugar chain(s) can be added may be a peptide comprising N-linked sugar chain(s)-added amino acid sequence. When the sugar chain(s) are O-linked sugar chain(s), the peptide to which the sugar chain(s) can be added may be a peptide comprising O-linked sugar chain(s)-added amino acid sequence. The present invention also provides a method for producing a heparin-binding protein functionalized by covalently bonding thereto sugar chain(s), comprising a step of allowing the sugar chain(s) to bind to the heparin-binding protein by a chemical binding method. The sugar chain(s) may be selected from the group consisting of sulfated polysaccharide(s), glycosaminoglycan(s), N-linked sugar chain(s), O-linked sugar chain(s) and a combination

thereof, and the heparin-binding protein may be a factor belonging to the FGF family or its allied factor. The present invention further provides a pharmaceutical composition containing, as an active ingredient, a heparin-binding protein functionalized by covalently bonding thereto sugar chain(s). The present invention also provides a method for functionalizing a natural protein having no sugar chain(s) by covalently bonding thereto sugar chain(s).

The novel sugar chain(s)-added heparin-binding protein of the invention is excellent in stabilities such as thermostability, acid resistance, alkali resistance and resistance to proteolytic enzymes. Thus, by using the sugar chain(s)-added heparin-binding protein of the invention in a pharmaceutical product, it is possible to design such a pharmaceutical product that is excellent in in vivo stabilities, in particular acid resistance and alkali resistance, and applicable to an oral medicine.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows typical examples of sulfated polysaccharide and glycosaminoglycan sugar chains.

FIG. 2 shows typical examples of N-linked sugar chains.

FIG. 3 shows typical examples of O-linked sugar chains.

FIG. 4A shows SDS-denatured electrophoregrams of S/FGF-1a-II Protein.

FIG. 4B shows SDS-denatured electrophoregrams of N-FGF-1a-IV and O-FGF-1a Proteins.

FIG. 5A shows the DNA synthesis promoting activity on HUVEC of S/FGF-1a-II.

FIG. 5B shows the DNA synthesis promoting activity on HUVEC of *E. coli*-derived FGF-1a.

FIG. 6A shows the thermostability, acid resistance and alkali resistance of S/FGF-1a-II.

FIG. 6B shows the thermostability, acid resistance and alkali resistance of *E. coli*-derived FGF-1a.

FIG. 7 shows the resistance to trypsin of S/FGF-1a-II and *E. coli*-derived FGF-1a.

FIG. 8 shows the DNA synthesis promoting activity on HUVEC of N-FGF-6/1a-IV and *E. coli*-derived FGF-1a.

FIG. 9 shows the heparin affinity of S/FGF-1a-II.

DESCRIPTION OF PREFERRED EMBODIMENTS

Hereinbelow, the present invention will be described in detail.

In the present invention, the heparin-binding protein to which sugar chain(s) are to be covalently bonded is a protein having heparin binding property. For example, factors belonging to the FGF family or allied factors, or other proteins with heparin-binding property but without structural similarity to the former proteins may be enumerated. Examples of the other proteins include, but are not limited to, heparin-binding epidermal growth factor-like factor (HB-EGF) and platelet-derived growth factor (PDGF). As specific examples of the factors belonging to the FGF family or allied factors, FGF-1 to -10 and FGF (fibroblast growth factor homologous factor)-1 to -4 are known. The heparin-binding protein of the invention may be covalently bonded to sugar chain(s) through a peptide to which the sugar chain(s) can be added. For example, the heparin-binding protein to which the sugar chain(s) are to be covalently bonded may be the following (a) or (b):

(a) a protein consisting of the amino acid sequence of SEQ ID NO: 1, 3, 5, 17, 19, 21, 23, 25, 27 or 29;

(b) a protein which consists of the amino acid sequence of SEQ ID NO: 1, 3, 5, 17, 19, 21, 23, 25, 27 or 29 having

deletion, substitution, addition or modification of one or several amino acids, which has FGF activity and to which the sugar chain(s) can be added.

Proteins having the amino acid sequences of SEQ ID NOS: 1, 3, 5, 17, 19, 21, 23, 25, 27 and 29 are encoded by, for example, the DNA sequences of SEQ ID NOS: 2, 4, 6, 18, 20, 22, 24, 26, 28 and 30, respectively. These proteins contain a peptide sequence to which sugar chain(s) can be added and a sequence for a signal peptide in addition to a peptide sequence for a factor belonging to the FGF family. The heparin-binding protein of the present invention includes not only the protein primarily defined by a cDNA shown in the sequence listing but also a protein in which a peptide sequence for secretion (called the signal peptide) located at the amino terminal when secreted from cells is cut off. The utility of a heparin-binding protein which is contained in the pharmaceutical composition of the invention as an active ingredient will not vary even if the protein is produced in a form lacking the signal peptide from the beginning.

The sugar chain(s) to be covalently bonded to the heparin-binding protein may be any sugar chain(s) as long as the protein is functionalized by covalently bonding the sugar chain(s). Examples of the sugar chain(s) include, but are not limited to, sulfated polysaccharides such as heparan sulfate, chondroitin sulfate, glycosaminoglycans, N-linked sugar chains and O-linked sugar chains. The term "functionalize" used herein means increasing the activity of a protein of interest. As an example of functionalization, there may be given a case in which the residual activity of a protein after treatment with heat, acid or alkali is increased by adding sugar chain(s) to the protein by covalent bond(s). The "sulfated polysaccharide(s)" used herein is a general term for various sugar chain structures which are elongating from xylose linked to a serine residue present in the primary structure of proteins or elongating on the non-reducing end side of N-linked sugar chains or O-linked sugar chains to be described later, or which are present in a free form.

Many of such sugar chains are composed of repeating disaccharides of aminosugar and uronic acid (or galactose), and some of their hydroxyl groups or amino groups are substituted with sulfate groups. Glycosaminoglycans are polysaccharides having a structure similar to those described above, but they include those which do not have any substitution with sulfate groups. All of the above-mentioned polysaccharides are designated herein generically "sulfated polysaccharides or the like".

Their specific structures are described, for example, in *Destiny of Sugar Chains in Cells*, Nagai, Hakomori and Kobata (Eds.), Kodansha Scientific Co. FIG. 1 shows their typical sugar chain sequences. The "N-linked sugar chain(s)" used herein is a general term for various sugar chain(s) structures elongating from N-acetylglucosamine linked to an asparagine residue present in the primary structure of proteins. Their specific structures are described, for example, in *Destiny of Sugar Chains in Cells*, Nagai, Hakomori and Kobata (Eds.), Kodansha Scientific Co. FIG. 2 shows their typical sugar chain sequences. The "O-linked sugar chain(s)" used herein is a general term for various sugar chain(s) structures elongating from N-acetylgalactosamine linked to a serine or threonine residue present in the primary structure of proteins. Their specific structures are described, for example, in *Destiny of Sugar Chains in Cells*, Nagai, Hakomori and Kobata (Eds.), Kodansha Scientific Co. FIG. 3 shows their typical sugar chain sequences. These sulfated polysaccharides or the like, N-linked sugar chains and O-linked sugar

chains may have addition, deletion, substitution or modification in a part of their sugar chain sequences as long as they retain their functions.

When sugar chain(s) are attached to a heparin-binding protein, the sugar chain(s) alone may be covalently bonded to the heparin-binding protein directly. Alternatively, a peptide chain of any length to which sugar chain(s) are covalently bonding may be covalently bonded to a heparin-binding protein.

In order to produce the heparin-binding protein of the invention to which sugar chain(s) are covalently bonded (hereinafter, referred to as the "sugar chain(s)-added heparin-binding protein"), first, a cDNA coding for a peptide to which sugar chain(s) can be added is ligated to a cDNA coding for a heparin-binding protein. The ligated cDNA is incorporated into an appropriate expression vector, which is then introduced into a host cell having sugar chain(s) addition pathway to thereby express sugar chain(s)-added heparin-binding protein.

cDNAs coding for various heparin-binding proteins can be obtained by designing appropriate primers from a sequence registered in a gene bank such as DDBJ (DNA Data Bank of Japan) and performing RT-PCR (reverse transcription PCR) with the primers and mRNA from the relevant tissue of the relevant animal.

In order to produce a sulfated polysaccharide or the like-added heparin-binding protein, first, a cDNA coding for a heparin-binding protein is ligated to a cDNA coding for a peptide which is known to undergo addition of a sulfated polysaccharide or the like. The ligated cDNA is incorporated into an appropriate host cell expression vector, which is then introduced into a host cell to thereby express the sulfated polysaccharide or the like-added heparin-binding protein. As the peptide which is known to undergo addition of a sulfated polysaccharide or the like, the core protein or a part thereof of various proteoglycans (e.g. syndecan, glypican, perlecan) may be used. As a part of the core protein of a proteoglycan, a peptide comprising a Ser-Gly repeat sequence (which is believed to be the sugar chain(s) addition site in proteoglycans) may be used.

In order to produce an N-linked sugar chain(s)-added heparin-binding protein, first, a cDNA coding for a heparin-binding protein is ligated to a cDNA coding for a peptide which is known to undergo addition of N-linked sugar chain(s). The ligated cDNA is incorporated into an appropriate host cell expression vector, which is then introduced into a host cell to thereby express the N-linked sugar chain(s)-added heparin-binding protein. Specific examples of the peptide which is known to undergo addition of N-linked sugar chain(s) include Asn-X-Thr and Asn-X-Ser (wherein X is any amino acid except proline).

In order to produce O-linked sugar chain(s)-added heparin-binding protein, first, a cDNA coding for a heparin-binding protein is ligated to a cDNA coding for a peptide which is known to undergo addition of O-linked sugar chain(s). The ligated cDNA is incorporated into an appropriate host cell expression vector, which is then introduced into a host cell to thereby express the O-linked sugar chain(s)-added heparin-binding protein. As a specific examples of the peptide which is known to undergo addition of O-linked sugar chain(s), Ala-Thr-Pro-Ala-Pro may be given.

As the site to which sugar chain(s) are bonded, a site forming a turn in the secondary structure of a heparin-binding protein or a site near one of the ends, or a site which would not change the tertiary structure of the protein greatly by addition of the sugar chain(s) is preferable.

One example of the method for producing sugar chain(s)-added heparin-binding protein of the invention will be described below.

First, an oligonucleotide coding for a secretion signal and a peptide which is known to undergo addition of sugar chain(s) is synthesized or amplified by PCR. The resultant oligonucleotide is incorporated at the 5' end of a plasmid coding for a heparin-binding protein.

As the secretion signal and the peptide which is known to undergo addition of sugar chain(s), an amino terminal of a typical secretion-type glycoprotein may be used, for example. Specifically, the amino acid consisting of the N terminal 40 residues of mouse FGF-6 may be used.

The plasmid coding for a heparin-binding protein can be prepared by incorporating a DNA coding for the heparin-binding protein into an appropriate plasmid. As the plasmid into which a DNA coding for a heparin-binding protein is to be incorporated, any plasmid may be used as long as it is replicated and maintained in a host. For example, pBR322 and pUC18 from *E. coli* and pET-3c which was constructed based on these plasmids may be enumerated.

As a method for incorporating the above-described oligonucleotide into the plasmid coding for a heparin-binding protein, the method described in T. Maniatis et al.: *Molecular Cloning*, Cold Spring Harbor Laboratory, p. 239 (1982) may be given, for example.

From the thus prepared plasmid, a region comprising a nucleotide sequence coding for a secretion signal, a peptide which is known to undergo addition of sugar chain(s) and a heparin-binding protein (hereinafter, referred to as a "region comprising a nucleotide sequence coding for sugar chain(s)-added heparin-binding protein") is cut out. This region is ligated to the downstream of a promoter in a vector suitable for expression to thereby obtain an expression vector.

The above-described region comprising a nucleotide sequence coding for sugar chain(s)-added heparin-binding protein may have ATG at its 5' end as a translation initiation codon and TAA, TGA or TAG at its 3' end as a translation termination codon. In order to express the protein encoded in the coding region, a promoter is ligated to the upstream of the region. As the promoter to be used in the present invention, any promoter may be used as long as it is appropriate to the host used for the expression of the gene. When the host to be transformed is a *bacillus*, SPO1 promoter, SPO2 promoter, penP promoter or the like may be used. When the host is a yeast, PHO5 promoter, PGK promoter, GAP promoter, ADH promoter or the like may be used. When the host is an animal cell, a promoter from SV40 or a promoter from a retrovirus may be used.

As the plasmid into which the thus constructed recombinant DNA comprising a nucleotide sequence coding for sugar chain(s)-added heparin-binding protein is to be incorporated, any plasmid may be used as long as it can be expressed in the host cell. For example, those vectors which were constructed based on *E. coli*-derived pBR322 and pUC18 may be given.

As a method for incorporating the recombinant DNA into a plasmid, the method described in T. Maniatis et al.: *Molecular Cloning*, Cold Spring Harbor Laboratory, p. 239 (1982) may be given, for example.

By introducing a vector comprising the above-described recombinant DNA into a host cell, a transformant carrying the vector is prepared.

As the host cell, any cell may be used as long as it has sugar chain(s) addition pathway. Specific examples include, but are not limited to, bacilli (e.g. *Bacillus subtilis* DB105), yeasts (e.g. *Pichia pastoris*, *Saccharomyces cerevisiae*), animal

cells (e.g. COS cell, CHO cell, BHK cell, NIH3T3 cell, BALB/c3T3 cell, HUVE cell, LEII cell) and insect cells (e.g. Sf-9 cell, Tn cell).

The above-mentioned transformation may be performed by a conventional method commonly used for each host. Alternatively, an applicable method may be used though it is not commonly used. For example, when the host is a yeast, a vector comprising the recombinant DNA is introduced into competent cells (prepared by the lithium method or the like) by the temperature shock method or electroporation. When the host is an animal cell, a vector comprising the recombinant DNA is introduced into cells at the logarithmic growth phase or the like by the calcium phosphate method, lipofection or electroporation.

By culturing the thus obtained transformant in a medium, a sugar chain(s)-added heparin-binding protein is produced. As the medium for culturing the transformant, a conventional medium commonly used for each host may be used. Alternatively, an applicable medium may be used even if it is not commonly used. For example, when the host is a yeast, YPD medium or the like may be used. When the host is an animal cell, Dulbecco's MEM supplemented with animal serum, or the like may be used. The cultivation may be performed under conditions commonly employed for each host. Alternatively, applicable conditions may be used even if they are not commonly used. For example, when the host is a yeast, the cultivation is carried out at about 25–37° C. for about 12 hours to 2 weeks. If necessary, aeration or agitation may be carried out. When the host is an animal cell, the cultivation is carried out at about 32–37° C. under 5% CO₂ and 100% humidity for about 24 hours to 2 weeks. If necessary, the conditions of the gas phase may be changed or agitation may be carried out.

In order to obtain a sugar-chain(s) added heparin-binding protein from the culture of the above-described transformant, the protein released into the culture fluid may be directly recovered from a supernatant after centrifugation. Alternatively, when the protein is to be extracted from the cultured microorganisms or cells, the protein may be obtained by disrupting the cultured microorganisms or cells with a homogenizer, a French press, ultrasonic waves, lysozyme and/or by freeze-thawing to thereby elute the protein of interest to the outside of the cells, and then recovering the protein from soluble fractions. If the protein of interest is contained in insoluble fractions, insoluble fractions may be recovered by centrifugation after disruption of the microorganisms or cells and then solubilized with a buffer containing guanidine hydrochloride or the like, to thereby recover the protein of interest from the resultant soluble fractions. Alternatively, the cultured microorganisms or cells may be disrupted by a direct treatment with a buffer containing a protein denaturing agent such as guanidine hydrochloride to thereby elute the protein of interest to the outside of the cells.

In order to purify a sugar chain(s)-added heparin-binding protein from the above-mentioned supernatant, known separation/purification methods may be used in an appropriate combination. Specific examples of these known separation/purification methods include salting out, solvent precipitation, dialysis, ultrafiltration, gel filtration, SDS-polyacrylamide gel electrophoresis, ion exchange chromatography, affinity chromatography, reversed phase high performance liquid chromatography and isoelectric focusing. Further, affinity chromatography using heparin sepharose as a carrier may be applicable to a large number of heparin-binding proteins.

The thus obtained sample may be dialyzed and freeze-dried to obtain dry powder if the activity of the sugar chain(s)-added heparin-binding protein is not damaged by

such processing. Further, in storing the sample, addition of serum albumin to the sample is effective for preventing adsorption of the sample to the container.

The inclusion of an extremely small amount of a reducing agent in the purification process or the storing process is preferable for preventing oxidation of the sample. As the reducing agent, β -mercaptoethanol, dithiothreitol, glutathione or the like may be used.

The sugar chain(s)-added heparin-binding protein of the invention may also be produced by attaching sugar chain(s) to a heparin-binding protein by a chemical method. As the specific method, the following a) or b), or a combination thereof may be used.

a) For example, first, sugar chain(s) are completed by a biological method, a chemical synthesis method or a combination thereof. At that time, a residue appropriate for protein binding may be introduced at one end of the sugar chain(s). For example, an aldehyde group is formed by reducing and partially oxidizing the reducing end of the completed sugar chain(s). Then, this aldehyde group is attached to an amino group in a protein by an amino bond to thereby complete the joining of the sugar chain(s) and the protein.

b) For example, first, an aldehyde group is formed by reducing and partially oxidizing the reducing end of a monosaccharide or a residue appropriate for protein binding which is bound to a monosaccharide. Then, this aldehyde group is attached to an amino group in a protein by an amino bond to thereby complete the joining of the monosaccharide and the protein. An additional monosaccharide or sugar chain(s) are attached to a hydroxyl group or the like of the above monosaccharide to thereby complete sugar chain(s). For this attachment, a biological method, a chemical synthesis method or a combination thereof may be considered.

A heparin-binding protein functionalized by covalently bonding thereto sugar chain(s) can be used as a medicine. For example, the sugar chain(s)-added heparin-binding protein of the invention regulates the physiological function of FGF. Specifically, the physiological function of FGF is to promote or inhibit the growth of fibroblast, vascular endothelial cell, myoblast, cartilage cell, osteoblast and glia cell. Therefore, the sugar chain(s)-added heparin-binding protein of the invention is effective for promoting cell growth and tissue regeneration in liver or the like; for curing wounds and regulating nervous function; and for regulating the growth of fibroblast or the like. The protein of the invention is useful for preventing or treating various diseases such as fibroblastoma, angioma, osteoblastoma, death of neurocytes, Alzheimer's disease, Parkinson's disease, neuroblastoma, amnesia, demensia and myocardial infarction. The protein of the invention can also be used as a trichogenous agent or a hair-growing agent.

The sugar chain(s)-added heparin-binding protein obtained as described above may be formulated into pharmaceutical compositions such as liquid, lotions, aerosols, injections, powder, granules, tablets, suppositories, enteric coated tablets and capsule, by mixing the protein with pharmaceutically acceptable solvents, vehicles, carriers, adjuvants, etc. according to conventional formulation methods.

The content of the sugar chain(s)-added heparin-binding protein, which is an active ingredient, in the pharmaceutical composition may be about 0.0000000001 to 1.0% by weight.

The pharmaceutical composition can be administered parenterally or orally to mammals, e.g. human, mouse, rat, rabbit, dog, cat, etc. in a safe manner. The dose of the pharmaceutical composition may be appropriately changed depending on the dosage form, administration route, conditions of the patient and the like. For example, for administra-

tion to mammals including human, 0.0001 to 100 mg of the sugar chain(s)-added heparin-binding protein may be applied to the diseased part several times a day.

The present invention has been described so far taking heparin-binding proteins as an example. However, it should be noted that besides the heparin-binding proteins, natural proteins having no sugar chain(s) can also be functionalized by covalently bonding thereto sugar chain(s).

Deposit of Microorganisms

Clones of *E. coli* DH5 α carrying plasmids incorporating genes coding for the sugar chain(s)-added heparin-binding proteins of the invention (having the DNA sequences of SEQ ID NOS: 2, 4, 18, 20, 22, 24, 26, 28 and 30, respectively) were deposited at the National Institute of Bioscience and Human-technology, Agency of Industrial Science and Technology under Accession Numbers of FERM BP-6428, FERM BP-6424, FERM BP-6427, FERM BP-6431, FERM BP-6429, FERM BP-6430, FERM BP-6423, FERM BP-1625 and FERM BP-6426 on Sep. 10, 1997.

Hereinbelow, the present invention will be described specifically with reference to the following Example. However, the present invention is not limited to this Examples.

EXAMPLE 1

1) Construction of S/FGF-1a-II Plasmid

1. Preparation of a Human Ryudocan cDNA Fragment

phR7A8 is a plasmid obtained by inserting a human ryudocan cDNA (PCR product) into the EcoR V site of pBluescript II (KS+) cloning vector. This plasmid contains a partial sequence from position 7 to position 2610 in the mRNA sequence shown under Accession No. D13292 (see B.B.R.C. Vol. 190, No. 3, pp. 814-822, 1993).

This plasmid was digested with Pvu II. Using the resultant DNA fragment of 2,232 base pairs as a template, a PCR (polymerase chain reaction) was performed. As primers, #109 (5'-TTG TCG ACC CAC CAT GGC CCC CGC CCG TCT-3') (SEQ ID NO: 7) and #111 (5'-TTG ATA TCT AGA GGC ACC AAG GGA TG-3') (SEQ ID NO: 8) were used. The specifically amplified 276 bp band was separated by electrophoresis. After extraction, this fragment was double-digested with EcoR V and Sal I. The resultant 268 bp band was separated, extracted and then used in the ligation described below.

2. FGF-1a/pBluescript II (KS+)

A PCR was performed using human FGF-1 cDNA as a template and #967 (5'-GCG TCG ACA GCG CTA ATT ACA AGA AGC CCA AAC TC-3') (SEQ ID NO: 9) and #630 (5'-CCG AAT TCG AAT TCT TTA ATC AGA AGA GAC TGG-3') (SEQ ID NO: 10) as primers. The specifically amplified 434 bp band was separated by electrophoresis. After extraction, this fragment was double-digested with EcoR I and Sal I. The resultant 422 bp band was separated, extracted and then inserted into pBluescript II (KS+) cloning vector (2934 bp) double-digested with EcoR I and Sal I, whereupon FGF-1a/pBluescript II (KS+) was produced.

FGF-1a/pBluescript II (KS+) was digested with Aor51H I and Sal I in this order. The resultant 2626 bp band was separated, extracted and then used in the ligation described below.

3. Preparation of S/FGF-1a-II Chimeric Gene EcoR V/Sal I fragment (a PCR product from human ryudocan) and Aor51H I/Sal I fragment from FGF-1a/pBluescript II (KS+) were subjected to a DNA ligation to produce S/FGF-1a-II/pBluescript II (KS+) vector. Subsequently, this vector was double-digested with EcoR I and Sal I to give a 678 bp band,

which was then separated and extracted. The resultant fragment was inserted into pMEXneo expression vector (5916 bp) double-digested with EcoR I and Sal I, whereupon S/FGF-1a-II/pMEXneo was produced. This expression vector comprises the nucleotide sequence shown in SEQ ID NO: 2.

2) Expression of S/FGF-1a-II

The resultant S/FGF-1a-II/pMEXneo was transferred into CHO-K1 cells (Chinese hamster ovary cell K1 substrain) by lipofection. Then, the cells were cultured in the presence of Geneticin to select gene-transferred cells. The selected cells were grown until the culture plate became almost full. Then, the medium was exchanged with a serum-free medium to increase the substance productivity of the cells. Thereafter, the medium was exchanged with a fresh one every two days. The resultant conditioned medium was subjected to low speed centrifugation, and the resultant supernatant was stored at 4° C.

3) Construction of N-FGF6/1a-IV Plasmid

1. Preparation of a Mouse FGF-6 cDNA Fragment

A PCR was performed using mouse FGF-6 cDNA as a template and #1048 (5'-GCG TCG ACC CAC CAT GTC CCG GGG AGC AGG ACG TGT TCA GGG CAC GCTGCA GGC TCT CGT CTT C-3') (SEQ ID NO: 11) and #968 (5'-GCG ATA TCC AGT AGC GTG CCG TTG GCG CG-3') (SEQ ID NO: 12) as primers. The specifically amplified 138 bp band was separated by electrophoresis. After extraction, this fragment was double-digested with EcoR V and Sal I. The resultant 130 bp band was separated, extracted and then used in the ligation described below.

2. Preparation of N-FGF6/1a-IV Chimeric Gene

EcoR V/Sal I fragment (a PCR product from mouse FGF-6) and Aor51H I/Sal I fragment from FGF-1a/pBluescript II (KS+) were subjected to a DNA ligation to produce N-FGF-6/1a-IV/pBluescript II (KS+) vector. Subsequently, this vector was double-digested with EcoR I and Sal I to give a 540 bp band, which was then separated and extracted. The resultant fragment was inserted into pMEXneo expression vector (5916 bp) double-digested with EcoR I and Sal I, whereupon N-FGF-6/1a-IV/pMEXneo was produced. This expression vector comprises the nucleotide sequence shown in SEQ ID NO: 4.

4) Expression of N-FGF-6/1a-IV

N-FGF-6/1a-IV was secreted into a culture supernatant by transferring N-FGF-6/1a-IV/pMEXneo into CHO-K1 cells in the same manner as described above for S/FGF6/1a-II.

5) Construction of O-FGF-6/1a Plasmid

1. Preparation of N-FGF6/1a<NQ> Chimeric Gene

A PCR was performed using N-FGF6/1a/pBluescript II (KS+) vector as a template and #105 (5'-GCG TCG ACC CAC CAT GTC-3') (SEQ ID NO: 13) and #124 (5'-GCG ATA TCC AGT AGC GTG CCT TGG GCG CG-3') (SEQ ID NO: 14) as primers. The specifically amplified 138 bp band was separated by electrophoresis. After extraction, this fragment was double-digested with EcoR V and Sal I. The resultant 130 bp band was subjected to the ligation described below together with Aor51H I/Sal I fragment from FGF-1a/pBluescript II (KS+), to thereby yield N-FGF-6/1a<NQ>/pBluescript II (KS+) vector.

2. Preparation of O-FGF-6/1a Chimeric Gene

A primary PCR was performed using N-FGF6/1a<NQ>/pBluescript II (KS+) vector as a template and #098 (5'-GCT GGA GGA GGC TGC TAC TCC AGC TTCAA CCA TTA CA-3') (SEQ ID NO: 15) and #116 (5'-GCC GCT CTA GAA CTA GTG GAT-3') (SEQ ID NO: 16) as primers. The

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specifically amplified 210 bp band was purified. Using this PCR product and #115 (5'-AAC AAA AGC TGG GTA CCG GG-3') as primers, a secondary PCR was performed. The specifically amplified 631 bp band was separated by electrophoresis. After extraction and purification, this fragment was double-digested with EcoR I and Sal I. The resultant 558 bp band was separated, extracted and then inserted into pMEX-neo expression vector (5916 bp) double-digested with EcoR I and Sal I, to thereby yield O-FGF-6/1a/pMEXneo. This expression vector comprises the nucleotide sequence shown in SEQ ID NO: 6.

6) Expression of O-FGF-6/1a

O-FGF-6/1a was secreted into a culture supernatant by transferring O-FGF6/1a/pMEXneo into CHO-K1 cells in the same manner as described above for S/FGF-1a-II.

7) Expression of FGF-1a in *E. coli*

The fragment from human FGF-1a cDNA obtained by double digestion with EcoR I and Sal I as described above was incorporated into an *E. coli* expression vector pET3c. *E. coli* BL21 (DE3)pLysS was transformed with the resultant vector. Subsequently, the transformant at the logarithmic growth phase was stimulated with IPTG (isopropylthio- β -galactoside) to induce the expression of the transferred gene. The cells were collected and sonicated for disruption to thereby release FGF-1a, which was then recovered in a centrifugation supernatant.

8) Removal of N-Linked Sugar Chains by Peptide N-Glycosidase F Treatment

N-FGF6/1a-II concentrated with heparin-Sepharose beads was boiled and eluted in an electrophoresis buffer, as will be described later (see Test Example 1). To a part of the resultant solution, NP-40 (final concentration: 1%), Tris-HCl buffer (pH 7.5) and peptide N-glycosidase F (0.3 U) were added and the mixture was kept at 37° C. overnight. Then, the solution was heated at 100° C. for 3 min to terminate the enzyme reaction. This reaction solution was analyzed by SDS-denatured electrophoresis, as will be described later.

Various S/FGF-1a and N-FGF-6/1a genes can be prepared by appropriately altering the PCR primers (#111 and #968) used in "1. Preparation of a Human Ryudocan cDNA Fragment" and "1. Preparation of a Mouse FGF-6 cDNA Fragment" in the above Example and by replacing the restriction enzyme EcoR V with an appropriate enzyme which would generate a blunt end. Examples of such cDNA sequences are shown in SEQ ID NOS: 8, 20, 22, 24, 26 and 28.

Various O-FGF-6/1a genes can be prepared by replacing the template used in the PCR in "2. Preparation of O-FGF-6/1a Chimeric Gene" above with S/FGF-1a-II/pBluescript II (KS+), N-FGF6/1a-IV/pBluescript II (KS+) or the like, or by appropriately altering the PCR primers (#098, #116 and #115), or by a combination of the both methods. An example of such a cDNA sequence is shown in SEQ ID NO: 30.

TEST EXAMPLE 1

SDS-Denatured Electrophoresis

Heparin Sepharose beads added to conditioned media of various FGF-1a-like proteins-secreting cells were individually washed and then boiled directly with an electrophoresis buffer (containing SDS and 2-¹ mercaptoethanol). The eluted protein was used as a sample. This sample was electrophoresed on 12.5% acrylamide gel in the presence of SDS and 2-mercaptoethanol. After being electrically transferred onto a nitrocellulose membrane, the protein was stained with anti-FGF-1 monoclonal antibody and horseradish peroxidase-la-

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belled anti-mouse IgG antibody, followed by detection by the chemiluminescence method (FIG. 4). In the Figure, the arrows at the left side indicate the locations of standard proteins with known molecular weights and their molecular weights (in daltons). Panel A) shows an SDS-denatured electrophoregram of S/FGF-1a-II. Panel B) shows SDS-denatured electrophoregrams of FGF-1a produced in *E. coli* (lane a); N-FGF-1a-IV obtained by treating N-FGF-6/1a-IV with peptide N-glycosidase F for removal of N-linked sugar chains (lane b); N-FGF6/1a-IV (lane c) and O-FGF-6/1a (lane d).

TEST EXAMPLE 2

DNA Synthesis Promoting Activity

The cell cycle of HUVEC (human umbilical cord-derived vascular endothelial cell) stops even in the presence of 15% serum if growth factors such as FGF are lacking. S/FGF-1a-II, N-FGF6/1a-IV, O-FGF-6/1a, or FGF-1a produced in *E. coli* was added to HUVEC in such a state. Eighteen hours later, radio-labelled thymidine was allowed to be taken up for 6 hours. The amount of radioactivity taken up into DNA during this period was regarded as indicating the amount of the newly synthesized DNA.

1. DNA Synthesis Promoting Effect (Heparin Non-Dependent) of S/FGF-1a-II on Human Vascular Endothelial Cell

A conditioned medium was prepared from a serum-free medium of S/FGF-1a-II gene-transferred cells. This conditioned medium was dialyzed against PBS and then added to HUVEC in the presence (5 μ g/ml) or absence of heparin, for examining the DNA synthesis promoting activity of S/FGF-1a-II on HUVEC (FIG. 5A). As a result, unlike FGF-1a produced in *E. coli* (FIG. 5B), S/FGF-1a-II promoted the DNA synthesis of HUVEC in a non-heparin-dependent manner (FIG. 5).

2. DNA Synthesis Promoting Effect of N-FGF6/1a-IV on Human Vascular Endothelial Cell

A conditioned medium was prepared from a serum-free medium of N-FGF-6/1a-IV gene-transferred cells. This conditioned medium was dialyzed against PBS and then added to HUVEC in the presence (5 μ g/ml) or absence of heparin, for examining the DNA synthesis promoting activity of N-FGF6/1a-IV on HUVEC. As a result, like FGF-1a produced in *E. coli*, N-FGF6/1a-IV promoted the DNA synthesis of HUVEC. However, its heparin dependency was weak, and N-FGF6/1a-IV exhibited stronger DNA synthesis promoting activity than FGF-1a from *E. coli* in the absence of heparin (FIG. 8).

TEST EXAMPLE 3

Heparin Affinity Chromatography

The heparin affinity of S/FGF-1a-II obtained in 2) in the above Example was examined. Heparin-Sepharose beads were added to a conditioned medium of S/FGF-1a-II-secreting cells and agitated at 4° C. for 2 hours or more. Beads precipitating by low speed centrifugation were recovered and washed sufficiently in physiological PBS (phosphate buffered saline, pH 7.4), followed by elution of the protein bound to heparin-fixed beads with PBS containing 2.5 M NaCl. After addition of distilled water to lower the salt concentration, this eluate was again applied to a high performance liquid chromatography column packed with heparin affinity beads. S/FGF-1a-II was eluted using NaCl density gradient.

While FGF-1a from *E. coli* was eluted at about 1.0 M NaCl, S/FGF-1a-II was eluted at about 0.4 M NaCl. Thus, it appears

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that affinity to the fixed heparin is lowered in S/FGF-1a-II (FIG. 9). The small peak seen around 1.0 M NaCl in FIG. 9 is considered to be a degradation product from S/FGF-1a-II as analyzed by SDS-denatured electrophoresis.

TEST EXAMPLE 4

Thermostability of FGF-1a-Like Proteins

Conditioned media of various FGF-1a-like protein-secreting cells were individually dialyzed against PBS sufficiently. A part of each of the resultant media was retained in PBS kept at 56° C. or 70° C. for 30 minutes, or retained at room temperature for 12 hours. Thereafter, the medium was re-dialyzed against PBS at 4° C. to prepare a sample. The stability of S/FGF-1a-II was determined by subjecting it to DNA synthesis promoting activity test on HUVEC after various treatments and then comparing the resultant activity with the activity of an S/FGF-1a-II sample dialyzed against PBS at 4° C. for 12 hours (FIG. 6A).

After retention at room temperature for 12 hours, even the activity of *E. coli*-derived FGF-1a was protected by heparin, but the activity of S/FGF-1a-II was protected regardless of the presence or absence of heparin (FIG. 6A).

After heat treatment at 56° C. for 30 minutes, *E. coli*-derived FGF-1a was almost deactivated, but S/FGF-1a-II retained about 50% of the activity. Thus, it was considered that its thermostability was improved (FIG. 6B).

TEST EXAMPLE 5

Acid Resistance and Alkali Resistance of FGF-1a-Like Proteins

Conditioned media of various FGF-1a-like protein-secreting cells were individually dialyzed against PBS sufficiently. A part of each of the resultant media was dialyzed in a citrate

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buffer (pH 4.0) or a sodium carbonate buffer (pH 10.0) for 12 hours and then re-dialyzed against PBS at 4° C. to prepare a sample. The stability of S/FGF-1a-II was determined by subjecting it to DNA synthesis promoting activity test on HUVEC after various treatments and then comparing the resultant activity with the activity of an S/FGF-1a-II sample dialyzed against PBS at 4° C. for 12 hours.

The activity of S/FGF-1a-II decreased little even after acid treatment at pH 4.0 regardless of the presence or absence of heparin; thus, an improvement in acid resistance was recognized (FIG. 6A). After alkali treatment at pH 10.0, *E. coli*-derived FGF-1a was almost deactivated, but S/FGF-1a-II retained about 50% of the activity; thus, an improvement was also recognized in alkali resistance (FIGS. 6A and 6B).

TEST EXAMPLE 6

Stability of FGF-1a-Like Proteins Against Proteolytic Enzymes

Conditioned media of various FGF-1a-like protein-secreting cells were individually dialyzed against PBS sufficiently. To a part of each of the resultant media, trypsin solutions of varying concentrations (0.0001–0.1%) were added and kept at 37° C. for 1 hour. The thus obtained sample was subjected to the SDS-denatured electrophoresis described previously. The intensity of the remaining band was compared to the intensity of the band generated by the sample before trypsin treatment to give an indicator of stability.

As a result, as shown in FIG. 7, 88% and 35% of the band intensity remained in S/FGF-1a-II after 0.001% and 0.01% trypsin treatment, respectively; however, the band intensity of *E. coli*-derived FGF-1a decreased to 58% and even to 6% after 0.001% and 0.01% trypsin treatment, respectively. Thus, it was considered that the resistance of S/FGF-1a-II to proteolytic enzymes was increased (FIG. 7).

SEQUENCE LISTING

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Arg Ala Asn Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu
 35          40          45

Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly
 50          55          60

Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln
 65          70          75          80

Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr
 85          90          95

Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln
100          105          110

Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His
115          120          125

Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val
130          135          140

Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr
145          150          155          160

Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
165          170          175

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<210> SEQ ID NO 4
<211> LENGTH: 525
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
sequence for a part of mouse fibroblast growth factor 6 and
a part of human fibroblast growth factor 1
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(525)

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<400> SEQUENCE: 4

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atg tcc cgg gga gca gga cgt gtt cag ggc acg ctg cag gct ctc gtc      48
Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val
 1           5           10           15

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ttc tta ggc gtc cta gtg ggc atg gtg gtg ccc tca cct gcc ggc gcc    96
Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala
      20              25              30

cgc gcc aac ggc acg cta ctg gac gct aat tac aag aag ccc aaa ctc    144
Arg Ala Asn Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu
      35              40              45

ctc tac tgt agc aac ggg ggc cac ttc ctg agg atc ctt ccg gat ggc    192
Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly
      50              55              60

aca gtg gat ggg aca agg gac agg agc gac cag cac att cag ctg cag    240
Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln
      65              70              75              80

ctc agt gcg gaa agc gtg ggg gag gtg tat ata aag agt acc gag act    288
Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr
      85              90              95

ggc cag tac ttg gcc atg gac acc gac ggg ctt tta tac gcc tca cag    336
Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln
      100             105             110

aca cca aat gag gaa tgt ttg ttc ctg gaa agg ctg gag gag aac cat    384
Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His
      115             120             125

tac aac acc tat ata tcc aag aag cat gca gag aag aat tgg ttt gtt    432
Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val
      130             135             140

ggc ctc aag aag aat ggg agc tgc aaa cgc ggt cct cgg act cac tat    480
Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr
      145             150             155             160

ggc cag aaa gca atc ttg ttt ctc ccc ctg cca gtc tct tct gat    525
Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
      165             170             175

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<210> SEQ ID NO 5

<211> LENGTH: 181

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
sequence for a part of mouse fibroblast growth factor 6,
a part of human fibroblast growth factor 1 and an artificial
sequence

<400> SEQUENCE: 5

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Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val
  1              5              10              15

Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala
  20              25              30

Arg Ala Gln Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu
  35              40              45

Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly
  50              55              60

Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln
  65              70              75              80

Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr
  85              90              95

Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln
  100             105             110

Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Ala Ala
  115             120             125

Thr Pro Ala Pro Asn His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala

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130	135	140	
Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg			
145	150	155	160
Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu			
	165	170	175
Pro Val Ser Ser Asp			
	180		
<210> SEQ ID NO 6			
<211> LENGTH: 543			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of			
sequence for a part of mouse fibroblast growth factor 6,			
a part of human fibroblast growth factor 1 and an artificial			
sequence			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(543)			
<400> SEQUENCE: 6			
atg tcc cgg gga gca gga cgt gtt cag ggc acg ctg cag gct ctc gtc			48
Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val			
1	5	10	15
ttc tta ggc gtc cta gtg ggc atg gtg gtg ccc tca cct gcc ggc gcc			96
Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala			
	20	25	30
cgc gcc caa ggc acg cta ctg gac gct aat tac aag aag ccc aaa ctc			144
Arg Ala Gln Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu			
	35	40	45
ctc tac tgt agc aac ggg ggc cac ttc ctg agg atc ctt ccg gat ggc			192
Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly			
	50	55	60
aca gtg gat ggg aca agg gac agg agc gac cag cac att cag ctg cag			240
Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln			
	65	70	80
ctc agt gcg gaa agc gtg ggg gag gtg tat ata aag agt acc gag act			288
Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr			
	85	90	95
ggc cag tac ttg gcc atg gac acc gac ggg ctt tta tac gcc tca cag			336
Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln			
	100	105	110
aca cca aat gag gaa tgt ttg ttc ctg gaa agg ctg gag gag gct gct			384
Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Ala Ala			
	115	120	125
act cca gct cca aac cat tac aac acc tat ata tcc aag aag cat gca			432
Thr Pro Ala Pro Asn His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala			
	130	135	140
gag aag aat tgg ttt gtt ggc ctc aag aag aat ggg agc tgc aaa cgc			480
Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg			
145	150	155	160
ggt cct cgg act cac tat ggc cag aaa gca atc ttg ttt ctc ccc ctg			528
Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu			
	165	170	175
cca gtc tct tct gat			543
Pro Val Ser Ser Asp			
	180		

<210> SEQ ID NO 7
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 7

ttgtgaccc accatggccc ccgcccgtct                               30

<210> SEQ ID NO 8
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 8

ttgatatacta gaggcaccaa gggatg                                   26

<210> SEQ ID NO 9
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 9

gcgtgacag cgctaattac aagaagocca aactc                           35

<210> SEQ ID NO 10
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 10

ccgaattcga attctttaat cagaagagac tgg                             33

<210> SEQ ID NO 11
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 11

gcgtgaccc accatgtccc ggggagcagg acgtgttcag ggcacgctgc aggtctctgt   60
cttc                                                                    64

<210> SEQ ID NO 12
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 12

gcgatatcca gtagcgtgcc gttggcgcg                                   29

<210> SEQ ID NO 13
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 13

gcgtcgaccc accatgtc                                     18

<210> SEQ ID NO 14
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 14

gcgatatcca gtagcgtgcc ttgggcgcg                       29

<210> SEQ ID NO 15
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 15

gctggaggag gctgtactc cagctccaaa ccattaca               38

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
      PCR

<400> SEQUENCE: 16

gccgctctag aactagtgga t                                21

<210> SEQ ID NO 17
<211> LENGTH: 200
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
      sequence for a part of human ryudocan and a part of human
      fibroblast growth factor 1

<400> SEQUENCE: 17

Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly
 1             5             10            15

Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu
 20            25            30

Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Pro Asp Asp Glu Asp Val
 35            40            45

Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly
 50            55            60

Asp Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly Gly
 65            70            75            80

His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg Asp
 85            90            95

Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val Gly

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100	105	110	
Glu Val Tyr Ile Lys Ser Thr	Glu Thr Gly Gln Tyr	Leu Ala Met Asp	
115	120	125	
Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu			
130	135	140	
Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys			
145	150	155	160
Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser			
165	170	175	
Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe			
180	185	190	
Leu Pro Leu Pro Val Ser Ser Asp			
195	200		
<210> SEQ ID NO 18			
<211> LENGTH: 600			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of			
sequence for a part of human ryudocan and a part of human			
fibroblast growth factor 1			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(600)			
<400> SEQUENCE: 18			
atg gcc ccc gcc cgt ctg ttc gcg ctg ctg ctg ttc ttc gta ggc gga			48
Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly			
1 5 10 15			
gtc gcc gag tcg atc cga gag act gag gtc atc gac ccc cag gac ctc			96
Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu			
20 25 30			
cta gaa ggc cga tac ttc tcc gga gcc cta cca gac gat gag gat gta			144
Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Pro Asp Asp Glu Asp Val			
35 40 45			
gtg ggg ccc ggg cag gaa tct gat gac ttt gag ctg tct ggc tct gga			192
Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly			
50 55 60			
gat gct aat tac aag aag ccc aaa ctc ctc tac tgt agc aac ggg ggc			240
Asp Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly Gly			
65 70 75 80			
cac ttc ctg agg atc ctt ccg gat ggc aca gtg gat ggg aca agg gac			288
His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg Asp			
85 90 95			
agg agc gac cag cac att cag ctg cag ctc agt gcg gaa agc gtg ggg			336
Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val Gly			
100 105 110			
gag gtg tat ata aag agt acc gag act ggc cag tac ttg gcc atg gac			384
Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu Ala Met Asp			
115 120 125			
acc gac ggg ctt tta tac ggc tca cag aca cca aat gag gaa tgt ttg			432
Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu			
130 135 140			
ttc ctg gaa agg ctg gag gag aac cat tac aac acc tat ata tcc aag			480
Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys			
145 150 155 160			
aag cat gca gag aag aat tgg ttt gtt ggc ctc aag aag aat ggg agc			528
Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser			
165 170 175			
tgc aaa cgc ggt cct cgg act cac tat ggc cag aaa gca atc ttg ttt			576

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Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe
 180 185 190

ctc ccc ctg cca gtc tct tct gat 600
 Leu Pro Leu Pro Val Ser Ser Asp
 195 200

<210> SEQ ID NO 19
 <211> LENGTH: 200
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of human ryudocan mutant and a part of human
 fibroblast growth factor 1

<400> SEQUENCE: 19

Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly
 1 5 10 15
 Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu
 20 25 30
 Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Ser Asp Asp Glu Asp Val
 35 40 45
 Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly
 50 55 60
 Asp Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly Gly
 65 70 75 80
 His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg Asp
 85 90 95
 Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val Gly
 100 105 110
 Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu Ala Met Asp
 115 120 125
 Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu
 130 135 140
 Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys
 145 150 155 160
 Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser
 165 170 175
 Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe
 180 185 190
 Leu Pro Leu Pro Val Ser Ser Asp
 195 200

<210> SEQ ID NO 20
 <211> LENGTH: 600
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of human ryudocan mutant and a part of human
 fibroblast growth factor 1
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(600)

<400> SEQUENCE: 20

atg gcc ccc gcc cgt ctg ttc gcg ctg ctg ctg ttc ttc gta ggc gga 48
 Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly
 1 5 10 15
 gtc gcc gag tcg atc cga gag act gag gtc atc gac ccc cag gac ctc 96
 Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu
 20 25 30

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cta gaa ggc cga tac ttc tcc gga gcc cta tca gac gat gag gat gta      144
Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Ser Asp Asp Glu Asp Val
      35                      40                      45

gtg ggg ccc ggg cag gaa tct gat gac ttt gag ctg tct ggc tct gga      192
Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly
      50                      55                      60

gat gct aat tac aag aag ccc aaa ctc ctc tac tgt agc aac ggg ggc      240
Asp Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly Gly
      65                      70                      75                      80

cac ttc ctg agg atc ctt ccg gat ggc aca gtg gat ggg aca agg gac      288
His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg Asp
      85                      90                      95

agg agc gac cag cac att cag ctg cag ctc agt gcg gaa agc gtg ggg      336
Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val Gly
      100                     105                     110

gag gtg tat ata aag agt acc gag act ggc cag tac ttg gcc atg gac      384
Glu Val Tyr Ile Lys Ser Thr Gly Thr Gly Gln Tyr Leu Ala Met Asp
      115                     120                     125

acc gac ggg ctt tta tac ggc tca cag aca cca aat gag gaa tgt ttg      432
Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu
      130                     135                     140

ttc ctg gaa agg ctg gag gag aac cat tac aac acc tat ata tcc aag      480
Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys
      145                     150                     155                     160

aag cat gca gag aag aat tgg ttt gtt ggc ctc aag aag aat ggg agc      528
Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser
      165                     170                     175

tgc aaa cgc ggt cct cgg act cac tat ggc cag aaa gca atc ttg ttt      576
Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe
      180                     185                     190

ctc ccc ctg cca gtc tct tct gat      600
Leu Pro Leu Pro Val Ser Ser Asp
      195                     200

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<210> SEQ ID NO 21

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
sequence for a part of human ryudocan and a part of human
fibroblast growth factor 1

<400> SEQUENCE: 21

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Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Phe Phe Val Gly Gly
  1                      5                      10                      15

Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu
      20                      25                      30

Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Pro Asp Asp Glu Asp Val
      35                      40                      45

Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly
      50                      55                      60

Asp Leu Asp Asp Leu Glu Asp Ser Met Ile Gly Pro Glu Val Val His
      65                      70                      75                      80

Pro Leu Val Pro Leu Asp Asn His Ile Pro Glu Arg Ala Gly Ser Gly
      85                      90                      95

Ser Gln Val Pro Thr Glu Pro Lys Lys Leu Glu Glu Asn Glu Val Ile
      100                     105                     110

Pro Lys Arg Ile Ser Pro Val Ala Asn Tyr Lys Lys Pro Lys Leu Leu

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115	120	125	
Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr			
130	135	140	
Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu			
145	150	155	160
Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly			
165	170	175	
Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr			
180	185	190	
Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr			
195	200	205	
Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly			
210	215	220	
Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly			
225	230	235	240
Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp			
245	250		
 <210> SEQ ID NO 22			
<211> LENGTH: 762			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of			
sequence for a part of human ryudocan and a part of human			
fibroblast growth factor 1			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(762)			
 <400> SEQUENCE: 22			
atg gcc ccc gcc cgt ctg ttc gcg ctg ctg ctg ttc ttc gta ggc gga			48
Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly			
1 5 10 15			
gtc gcc gag tcg atc cga gag act gag gtc atc gac ccc cag gac ctc			96
Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu			
20 25 30			
cta gaa ggc cga tac ttc tcc gga gcc cta cca gac gat gag gat gta			144
Leu Glu Gly Arg Tyr Phe Ser Ser Gly Ala Leu Pro Asp Asp Glu Asp Val			
35 40 45			
gtg ggg ccc ggg cag gaa tct gat gac ttt gag ctg tct ggc tct gga			192
Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly			
50 55 60			
gat ctg gat gac ttg gaa gac tcc atg atc ggc cct gaa gtt gtc cat			240
Asp Leu Asp Asp Leu Glu Asp Ser Met Ile Gly Pro Glu Val Val His			
65 70 75 80			
ccc ttg gtg cct cta gat aac cat atc cct gag agg gca ggg tct ggg			288
Pro Leu Val Pro Leu Asp Asn His Ile Pro Glu Arg Ala Gly Ser Gly			
85 90 95			
agc caa gtc ccc acc gaa ccc aag aaa cta gag gag aat gag gtt atc			336
Ser Gln Val Pro Thr Glu Pro Lys Lys Leu Glu Glu Asn Glu Val Ile			
100 105 110			
ccc aag aga atc tca ccc gtt gct aat tac aag aag ccc aaa ctc ctc			384
Pro Lys Arg Ile Ser Pro Val Ala Asn Tyr Lys Lys Pro Lys Leu Leu			
115 120 125			
tac tgt agc aac ggg ggc cac ttc ctg agg atc ctt ccg gat ggc aca			432
Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr			
130 135 140			
gtg gat ggg aca agg gac agg agc gac cag cac att cag ctg cag ctc			480
Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu			
145 150 155 160			

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agt gcg gaa agc gtg ggg gag gtg tat ata aag agt acc gag act ggc      528
Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly
      165                      170                      175

cag tac ttg gcc atg gac acc gac ggg ctt tta tac ggc tca cag aca      576
Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr
      180                      185                      190

cca aat gag gaa tgt ttg ttc ctg gaa agg ctg gag gag aac cat tac      624
Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr
      195                      200                      205

aac acc tat ata tcc aag aag cat gca gag aag aat tgg ttt gtt ggc      672
Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly
      210                      215                      220

ctc aag aag aat ggg agc tgc aaa cgc ggt cct cgg act cac tat ggc      720
Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly
      225                      230                      235                      240

cag aaa gca atc ttg ttt ctc ccc ctg cca gtc tct tct gat      762
Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
      245                      250

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<210> SEQ ID NO 23
<211> LENGTH: 281
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
sequence for a part of human ryudocan and a part of human
fibroblast growth factor 1

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<400> SEQUENCE: 23

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Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Leu Phe Phe Val Gly Gly
  1              5              10              15

Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu
  20              25              30

Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Pro Asp Asp Glu Asp Val
  35              40              45

Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly
  50              55              60

Asp Leu Asp Asp Leu Glu Asp Ser Met Ile Gly Pro Glu Val Val His
  65              70              75              80

Pro Leu Val Pro Leu Asp Asn His Ile Pro Glu Arg Ala Gly Ser Gly
  85              90              95

Ser Gln Val Pro Thr Glu Pro Lys Lys Leu Glu Glu Asn Glu Val Ile
  100             105             110

Pro Lys Arg Ile Ser Pro Val Glu Glu Ser Glu Asp Val Ser Asn Lys
  115             120             125

Val Ser Met Ser Ser Thr Val Gln Gly Ser Asn Ile Phe Glu Arg Thr
  130             135             140

Glu Val Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly
  145             150             155             160

Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg
  165             170             175

Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val
  180             185             190

Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu Ala Met
  195             200             205

Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys
  210             215             220

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Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser
 225 230 235 240

Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly
 245 250 255

Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu
 260 265 270

Phe Leu Pro Leu Pro Val Ser Ser Asp
 275 280

<210> SEQ ID NO 24
 <211> LENGTH: 843
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of human ryudocan and a part of human
 fibroblast growth factor 1
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(843)

<400> SEQUENCE: 24

atg gcc ccc gcc cgt ctg ttc gcg ctg ctg ttc ttc gta ggc gga Met Ala Pro Ala Arg Leu Phe Ala Leu Leu Phe Phe Val Gly Gly 1 5 10 15	48
gtc gcc gag tcg atc cga gag act gag gtc atc gac ccc cag gac ctc Val Ala Glu Ser Ile Arg Glu Thr Glu Val Ile Asp Pro Gln Asp Leu 20 25 30	96
cta gaa ggc cga tac ttc tcc gga gcc cta cca gac gat gag gat gta Leu Glu Gly Arg Tyr Phe Ser Gly Ala Leu Pro Asp Asp Glu Asp Val 35 40 45	144
gtg ggg ccc ggg cag gaa tct gat gac ttt gag ctg tct ggc tct gga Val Gly Pro Gly Gln Glu Ser Asp Asp Phe Glu Leu Ser Gly Ser Gly 50 55 60	192
gat ctg gat gac ttg gaa gac tcc atg atc ggc cct gaa gtt gtc cat Asp Leu Asp Asp Leu Glu Asp Ser Met Ile Gly Pro Glu Val Val His 65 70 75 80	240
ccc ttg gtg cct cta gat aac cat atc cct gag agg gca ggg tct ggg Pro Leu Val Pro Leu Asp Asn His Ile Pro Glu Arg Ala Gly Ser Gly 85 90 95	288
agc caa gtc ccc acc gaa ccc aag aaa cta gag gag aat gag gtt atc Ser Gln Val Pro Thr Glu Pro Lys Lys Leu Glu Glu Asn Glu Val Ile 100 105 110	336
ccc aag aga atc tca ccc gtt gaa gag agt gag gat gtg tcc aac aag Pro Lys Arg Ile Ser Pro Val Glu Glu Ser Glu Asp Val Ser Asn Lys 115 120 125	384
gtg tca atg tcc agc act gtg cag ggc agc aac atc ttt gag aga acg Val Ser Met Ser Ser Thr Val Gln Gly Ser Asn Ile Phe Glu Arg Thr 130 135 140	432
gag gtc gct aat tac aag aag ccc aaa ctc ctc tac tgt agc aac ggg Glu Val Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn Gly 145 150 155 160	480
ggc cac ttc ctg agg atc ctt ccg gat ggc aca gtg gat ggg aca agg Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Arg 165 170 175	528
gac agg agc gac cag cac att cag ctg cag ctc agt gcg gaa agc gtg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser Val 180 185 190	576
ggg gag gtg tat ata aag agt acc gag act ggc cag tac ttg gcc atg Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu Ala Met 195 200 205	624
gac acc gac ggg ctt tta tac ggc tca cag aca cca aat gag gaa tgt	672

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Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys	
210 215 220	
ttg ttc ctg gaa agg ctg gag gag aac cat tac aac acc tat ata tcc	720
Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser	
225 230 235 240	
aag aag cat gca gag aag aat tgg ttt gtt ggc ctc aag aag aat ggg	768
Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly	
245 250 255	
agc tgc aaa cgc ggt cct cgg act cac tat ggc cag aaa gca atc ttg	816
Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu	
260 265 270	
ttt ctc ccc ctg cca gtc tct tct gat	843
Phe Leu Pro Leu Pro Val Ser Ser Asp	
275 280	

<210> SEQ ID NO 25
 <211> LENGTH: 172
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of mouse fibroblast growth factor 6 and
 a part of human fibroblast growth factor 1

<400> SEQUENCE: 25

Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val	
1 5 10 15	
Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala	
20 25 30	
Arg Ala Asn Gly Ser Ala Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys	
35 40 45	
Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp	
50 55 60	
Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala	
65 70 75 80	
Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr	
85 90 95	
Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn	
100 105 110	
Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr	
115 120 125	
Tyr Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys	
130 135 140	
Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys	
145 150 155 160	
Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp	
165 170	

<210> SEQ ID NO 26
 <211> LENGTH: 516
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of mouse fibroblast growth factor 6 and
 a part of human fibroblast growth factor 1
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(516)

<400> SEQUENCE: 26

atg tcc cgg gga gca gga cgt gtt cag ggc acg ctg cag gct ctc gtc	48
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Met	Ser	Arg	Gly	Ala	Gly	Arg	Val	Gln	Gly	Thr	Leu	Gln	Ala	Leu	Val	
1				5					10					15		
ttc	tta	ggc	gtc	cta	gtg	ggc	atg	gtg	gtg	ccc	tca	cct	gcc	ggc	gcc	96
Phe	Leu	Gly	Val	Leu	Val	Gly	Met	Val	Val	Pro	Ser	Pro	Ala	Gly	Ala	
			20					25					30			
cgc	gcc	aac	ggc	tcg	gct	aat	tac	aag	aag	ccc	aaa	ctc	ctc	tac	tgt	144
Arg	Ala	Asn	Gly	Ser	Ala	Asn	Tyr	Lys	Lys	Pro	Lys	Leu	Leu	Tyr	Cys	
		35				40						45				
agc	aac	ggg	ggc	cac	ttc	ctg	agg	atc	ctt	cgg	gat	ggc	aca	gtg	gat	192
Ser	Asn	Gly	Gly	His	Phe	Leu	Arg	Ile	Leu	Pro	Asp	Gly	Thr	Val	Asp	
	50				55				60							
ggg	aca	agg	gac	agg	agc	gac	cag	cac	att	cag	ctg	cag	ctc	agt	gcg	240
Gly	Thr	Arg	Asp	Arg	Ser	Asp	Gln	His	Ile	Gln	Leu	Gln	Leu	Ser	Ala	
	65			70					75					80		
gaa	agc	gtg	ggg	gag	gtg	tat	ata	aag	agt	acc	gag	act	ggc	cag	tac	288
Glu	Ser	Val	Gly	Glu	Val	Tyr	Ile	Lys	Ser	Thr	Glu	Thr	Gly	Gln	Tyr	
			85					90					95			
ttg	gcc	atg	gac	acc	gac	ggg	ctt	tta	tac	ggc	tca	cag	aca	cca	aat	336
Leu	Ala	Met	Asp	Thr	Asp	Gly	Leu	Tyr	Gly	Ser	Gln	Thr	Pro	Asn		
		100					105					110				
gag	gaa	tgt	ttg	ttc	ctg	gaa	agg	ctg	gag	gag	aac	cat	tac	aac	acc	384
Glu	Glu	Cys	Leu	Phe	Leu	Glu	Arg	Leu	Glu	Glu	Asn	His	Tyr	Asn	Thr	
		115				120					125					
tat	ata	tcc	aag	aag	cat	gca	gag	aag	aat	tgg	ttt	gtt	ggc	ctc	aag	432
Tyr	Ile	Ser	Lys	Lys	His	Ala	Glu	Lys	Asn	Trp	Phe	Val	Gly	Leu	Lys	
	130				135					140						
aag	aat	ggg	agc	tcg	aaa	cgc	ggt	cct	cgg	act	cac	tat	ggc	cag	aaa	480
Lys	Asn	Gly	Ser	Cys	Lys	Arg	Gly	Pro	Arg	Thr	His	Tyr	Gly	Gln	Lys	
	145			150				155						160		
gca	atc	ttg	ttt	ctc	ccc	ctg	cca	gtc	tct	tct	gat					516
Ala	Ile	Leu	Phe	Leu	Pro	Leu	Pro	Val	Ser	Ser	Asp					
			165					170								

<210> SEQ ID NO 27

<211> LENGTH: 210

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
sequence for a part of mouse fibroblast growth factor 6 and
a part of human fibroblast growth 1

<400> SEQUENCE: 27

Met	Ser	Arg	Gly	Ala	Gly	Arg	Val	Gln	Gly	Thr	Leu	Gln	Ala	Leu	Val	
1				5					10					15		
Phe	Leu	Gly	Val	Leu	Val	Gly	Met	Val	Val	Pro	Ser	Pro	Ala	Gly	Ala	
			20					25					30			
Arg	Ala	Asn	Gly	Thr	Leu	Leu	Asp	Ser	Arg	Gly	Trp	Gly	Thr	Leu	Leu	
		35					40					45				
Ser	Arg	Ser	Arg	Ala	Gly	Leu	Ala	Gly	Glu	Ile	Ser	Gly	Val	Asn	Trp	
	50				55				60							
Glu	Ser	Gly	Tyr	Leu	Val	Gly	Ile	Lys	Arg	Gln	Ala	Asn	Tyr	Lys	Lys	
	65			70					75					80		
Pro	Lys	Leu	Leu	Tyr	Cys	Ser	Asn	Gly	Gly	His	Phe	Leu	Arg	Ile	Leu	
			85					90					95			
Pro	Asp	Gly	Thr	Val	Asp	Gly	Thr	Arg	Asp	Arg	Ser	Asp	Gln	His	Ile	
		100					105						110			
Gln	Leu	Gln	Leu	Ser	Ala	Glu	Ser	Val	Gly	Glu	Val	Tyr	Ile	Lys	Ser	
		115					120					125				

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Thr Glu Thr Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr
 130 135 140

Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu
 145 150 155 160

Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn
 165 170 175

Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg
 180 185 190

Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser
 195 200 205

Ser Asp
 210

<210> SEQ ID NO 28
 <211> LENGTH: 630
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of mouse fibroblast growth factor 6 and
 a part of human fibroblast growth 1
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(630)

<400> SEQUENCE: 28

atg tcc cgg gga gca gga cgt gtt cag ggc acg ctg cag gct ctc gtc	48
Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val	
1 5 10 15	
ttc tta ggc gtc cta gtg ggc atg gtg gtg ccc tca cct gcc ggc gcc	96
Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala	
20 25 30	
cgc gcc aac ggc acg cta ctg gac tcc aga ggc tgg ggc acc ctc ttg	144
Arg Ala Asn Gly Thr Leu Leu Asp Ser Arg Gly Trp Gly Thr Leu Leu	
35 40 45	
tcc agg tct cga gct ggg cta gct gga gag att tcg ggt gtg aat tgg	192
Ser Arg Ser Arg Ala Gly Leu Ala Gly Glu Ile Ser Gly Val Asn Trp	
50 55 60	
gaa agc ggc tat ttg gtg ggc att aag cga cag gct aat tac aag aag	240
Glu Ser Gly Tyr Leu Val Gly Ile Lys Arg Gln Ala Asn Tyr Lys Lys	
65 70 75 80	
ccc aaa ctc ctc tac tgt agc aac ggg ggc cac ttc ctg agg atc ctt	288
Pro Lys Leu Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu	
85 90 95	
cgg gat ggc aca gtg gat ggg aca agg gac agg agc gac cag cac att	336
Pro Asp Gly Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile	
100 105 110	
cag ctg cag ctc agt gcg gaa agc gtg ggg gag gtg tat ata aag agt	384
Gln Leu Gln Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser	
115 120 125	
acc gag act ggc cag tac ttg gcc atg gac acc gac ggg ctt tta tac	432
Thr Glu Thr Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr	
130 135 140	
ggc tca cag aca cca aat gag gaa tgt ttg ttc ctg gaa agg ctg gag	480
Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu	
145 150 155 160	
gag aac cat tac aac acc tat ata tcc aag aag cat gca gag aag aat	528
Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys Asn	
165 170 175	
tgg ttt gtt ggc ctc aag aag aat ggg agc tgc aaa cgc ggt cct cgg	576
Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly Pro Arg	
180 185 190	

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act cac tat ggc cag aaa gca atc ttg ttt ctc ccc ctg cca gtc tct 624
 Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser
 195 200 205

tct gat 630
 Ser Asp
 210

<210> SEQ ID NO 29
 <211> LENGTH: 180
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of mouse fibroblast growth factor 6,
 a part of human fibroblast growth factor 1 and an artificial
 sequence

<400> SEQUENCE: 29

Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val
 1 5 10 15
 Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala
 20 25 30
 Arg Ala Asn Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu
 35 40 45
 Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly
 50 55 60
 Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln
 65 70 75 80
 Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr
 85 90 95
 Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln
 100 105 110
 Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn Ala
 115 120 125
 Thr Pro Ala Pro His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu
 130 135 140
 Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly
 145 150 155 160
 Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro
 165 170 175
 Val Ser Ser Asp
 180

<210> SEQ ID NO 30
 <211> LENGTH: 540
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: fusion of
 sequence for a part of mouse fibroblast growth factor 6,
 a part of human fibroblast growth factor 1 and an artificial
 sequence
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(540)
 <400> SEQUENCE: 30

atg tcc cgg gga gca gga cgt gtt cag ggc acg ctg cag gct ctc gtc 48
 Met Ser Arg Gly Ala Gly Arg Val Gln Gly Thr Leu Gln Ala Leu Val
 1 5 10 15
 ttc tta ggc gtc cta gtg ggc atg gtg gtg ccc tca cct gcc gcc gcc 96
 Phe Leu Gly Val Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Ala

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20	25	30	
cgc gcc aac ggc acg cta ctg gac gct aat tac aag aag ccc aaa ctc			144
Arg Ala Asn Gly Thr Leu Leu Asp Ala Asn Tyr Lys Lys Pro Lys Leu			
35	40	45	
ctc tac tgt agc aac ggg ggc cac ttc ctg agg atc ctt ccg gat ggc			192
Leu Tyr Cys Ser Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly			
50	55	60	
aca gtg gat ggg aca agg gac agg agc gac cag cac att cag ctg cag			240
Thr Val Asp Gly Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln			
65	70	75	80
ctc agt gcg gaa agc gtg ggg gag gtg tat ata aag agt acc gag act			288
Leu Ser Ala Glu Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr			
85	90	95	
ggc cag tac ttg gcc atg gac acc gac ggg ctt tta tac ggc tca cag			336
Gly Gln Tyr Leu Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln			
100	105	110	
aca cca aat gag gaa tgt ttg ttc ctg gaa agg ctg gag gag aac gct			384
Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn Ala			
115	120	125	
act cca gct cca cat tac aac acc tat ata tcc aag aag cat gca gag			432
Thr Pro Ala Pro His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu			
130	135	140	
aag aat tgg ttt gtt ggc ctc aag aag aat ggg agc tgc aaa cgc ggt			480
Lys Asn Trp Phe Val Gly Leu Lys Lys Asn Gly Ser Cys Lys Arg Gly			
145	150	155	160
cct cgg act cac tat ggc cag aaa gca atc ttg ttt ctc ccc ctg cca			528
Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro			
165	170	175	
gtc tct tct gat			540
Val Ser Ser Asp			
180			
 <210> SEQ ID NO 31			
<211> LENGTH: 20			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:primer for			
PCR			
 <400> SEQUENCE: 31			
aacaaaagct gggtaccggg			

20

What is claimed is:

1. A functionalized heparin-binding protein comprising a heparin-binding protein and at least one sugar chain covalently bonded thereto, said at least one covalently bonded sugar chain being selected from the group consisting of a sulfated polysaccharide, a glycosaminoglycan and an O-linked sugar chain, said heparin-binding protein comprising (a) a proteoglycan core protein or a part thereof, to which said sugar chain is bonded, and (b) the portion of the amino acid sequence of SEQ ID NO: 1 starting with Asn at number 88 and ending with Asp at number 221, wherein the DNA synthesis promoting activity of the heparin-binding protein is increased by adding the at least one covalently bonded sugar chain.

2. The functionalized heparin-binding protein of claim 1, wherein the at least one sugar chain is heparan sulfate.

3. The functionalized heparin-binding protein of claim 1, wherein the functionalized heparin-binding protein has improved stability over an unmodified heparin-binding protein.

4. The functionalized heparin-binding protein of claim 3, wherein the stability is chosen from among the group consisting of thermostability, acid resistance, alkalai resistance and resistance to proteolytic enzymes.

5. A pharmaceutical composition containing the functionalized heparin-binding protein of claim 1 as an active ingredient.

* * * * *